

Stereochemical studies. VI

Steric relations of some α -arylpropionic acids

By BERNDT SJÖBERG

With 18 figures in the text

In previous papers the configuration of some α -arylpropionic acids as well as the plant growth regulating activities of the enantiomers have been reported (1-7). The purpose of these investigations has been to establish whether there is a general connection between auxin activity and steric configuration of optically active plant growth regulators. It has been shown by Matell that for a series of α -aryloxyalkyl carboxylic acids the D-forms are more active than the corresponding L-forms (8).

For all the acids referred to but one, α -(2-naphthyl)-propionic acid (IV), the configuration has previously been established and reported as well as preliminary experiments on the plant growth regulating activity (1-7). This paper will review the results for the following propionic acids: α -phenyl- (I), α -(1-naphthyl)- (II), α -(4-methyl-1-naphthyl)- (III), α -(2-naphthyl)- (IV), α -(2-thianaphthenyl)- (V), α -(3-thianaphthenyl)- (VI), and α -(3-indolyl)-propionic acid (VII).

Steric relations

The steric relations between the investigated acids are given in Fig. 1. All of the compounds are dextrorotatory in absolute ethanol and are represented by the stereoformulas I-IX, according to the Fischer convention. The solid arrows indicate relations obtained by the Fredga method (9) and the dashed arrows refer to chemical reactions not involving the asymmetric center.

The Fredga method (quasi-racemate method), chemical and optical methods were used when establishing the steric relations. The quasi-racemate method was extremely useful in this series and gave information not only about steric relations, but it also gave indications of structural similarities between some of the acids. The method is based on the fact that one component in a true racemate may be replaced by a similar compound with the same configuration. The structural differences between the components must not be too great in order to get a quasi-racemate. Formation of a quasi-racemate is also more probable when one of the components or both form racemates of great stability (9, 10, 11). The existence of the molecular compounds was demonstrated by thermal analysis and in uncertain cases X-ray powder photographs were taken.

As can be seen in Fig. 1 it was possible to connect α -(1-naphthyl)- (II) and α -(2-

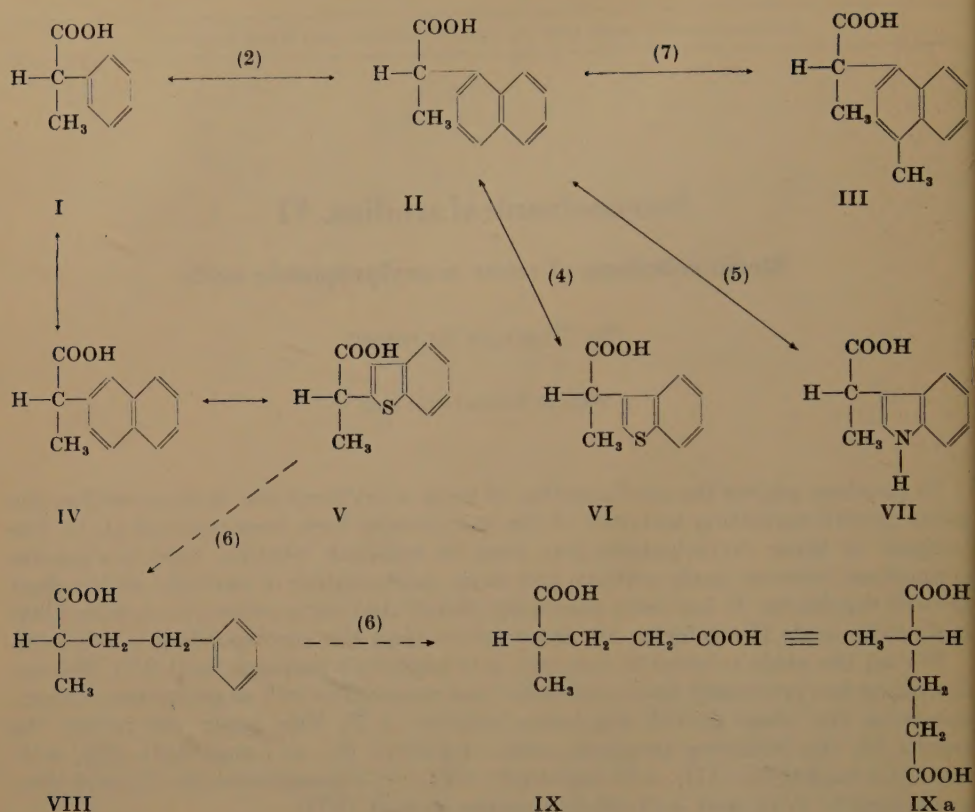


Fig. 1. Sterically related acids. All of the compounds are dextrorotatory in ethanol.

naphthyl)-propionic acid (IV) via hydratropic acid (I). The configuration of hydratropic acid has been investigated by many authors using different methods, and there was very strong evidence that (+)-hydratropic acid belonged to the D-glyceraldehyde system (1, 12, 13).

Fredga succeeded in relating α -(1-naphthyl)-propionic acid to hydratropic acid. The acids with opposite direction of rotation formed a quasi-racemate (2). The melting point diagram is reproduced in Fig. 8. It was pointed out that the existence of the quasi-racemic compound in some way must be connected with the remarkable stability of the racemate of α -(1-naphthyl)-propionic acid (Fig. 2).

α -(2-Naphthyl)-propionic acid (IV) forms a racemate of fairly low stability (Fig. 4), but still a quasi-racemic compound was formed with hydratropic acid having the opposite direction of rotation (Fig. 9). X-ray investigations gave further evidence for a molecular compound between (+)- α -(2-naphthyl)-propionic acid and (-)-hydratropic acid. The melting point curve for the system containing acids with the same direction of rotation was of simple eutectic type (Fig. 10). Consequently, the acids with the same direction of rotation (measured in ethanol) also have the same configuration.

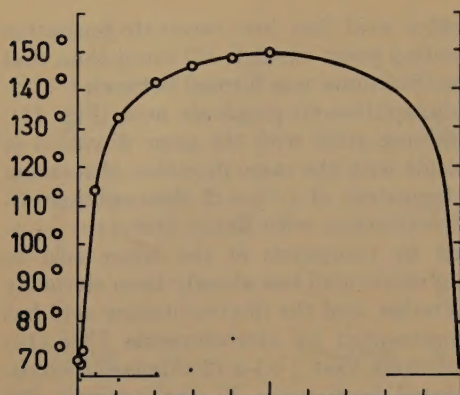
It was furthermore possible to connect α -(2-naphthyl)-propionic acid to α -(2-

thianaphthenyl)-propionic acid (V). The latter acid has low racemate-formation tendency and the racemic compound has a melting point which is 17° lower than that for the antipodes (Fig. 5). Nevertheless a quasi-racemate was formed between $(-)\text{-}\alpha\text{-(2-naphthyl)-propionic acid}$ and $(+)\text{-}\alpha\text{-(2-thianaphthenyl)-propionic acid}$ (Fig. 11). The melting point curve for the system containing acids with the same direction of rotation was of eutectic type (Fig. 12). The acids with the same direction of rotation thus have the same configuration. The configuration of $(+)\text{-}\alpha\text{-(2-thianaphthenyl)-propionic acid}$ has been established by desulphurization with Raney nickel to $(+)\text{-}\alpha\text{-methyl-}\gamma\text{-phenylbutyric acid}$ (VIII) followed by ozonolysis of the latter acid to $(+)\text{-}\alpha\text{-methylglutaric acid}$ (IX) (6). $\alpha\text{-Methylglutaric acid}$ has already been sterically correlated to the glyceraldehyde system by Fredga, and the dextrorotatory acid has been shown to have the configuration represented by stereoformula IX a (14) which is identical with IX. It is thus obvious that $(+)\text{-}\alpha\text{-(2-thianaphthenyl)-propionic acid}$ has a configuration represented by formula V. Consequently the dextrorotatory hydratropic acid is represented by formula I and the earlier evidence for the D-configuration of $(+)\text{-hydratropic acid}$ has also been proved to be correct.

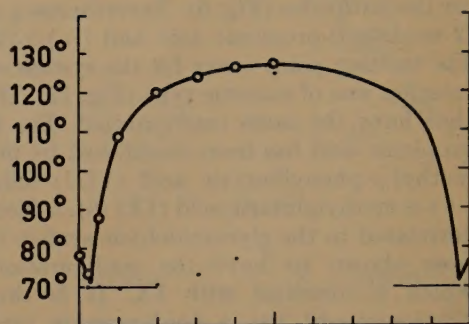
In a previous paper (4), it was shown that the configuration of $\alpha\text{-(3-thianaphthenyl)-propionic acid}$ (VI) could be related to $\alpha\text{-(1-naphthyl)-propionic acid}$ (II). The two acids have very great racemate-formation tendency (Figs. 2 and 6), and a very pronounced quasi-racemate was formed between the acids with opposite direction of rotation (Fig. 13). The acids with the same direction of rotation showed mutual solubility in the solid state (Fig. 14). As mentioned above a quasi-racemate was formed between $\alpha\text{-(2-naphthyl)-propionic acid}$ (IV) and $\alpha\text{-(2-thianaphthenyl)-propionic acid}$ (V). Experiments according to the Kofler contact method (15) gave no evidence of molecular compounds between $\alpha\text{-(1-naphthyl)-}$ and $\alpha\text{-(2-naphthyl)-propionic acid}$, between $\alpha\text{-(1-naphthyl)-}$ and $\alpha\text{-(2-thianaphthenyl)-propionic acid}$, nor between $\alpha\text{-(2-thianaphthenyl)-}$ and $\alpha\text{-(3-thianaphthenyl)-propionic acid}$. These experiments indicate that the 1-position in naphthalene is structurally similar to the 3-position in thianaphthene and that the 2-positions also are comparable. The sulphur atom in thianaphthene is structurally similar to a $-\text{CH}=\text{CH}-$ group in naphthalene. An analogous relation has been found between benzene and thiophene (16).

$\alpha\text{-(1-Naphthyl)-propionic acid}$ was also used for establishing the configuration of $\alpha\text{-(3-indolyl)-propionic acid}$ (VII) (5). This acid has a very low racemate formation tendency (Fig. 7). The difference in melting point between the optically active $\alpha\text{-(1-naphthyl)-propionic acid}$ and $\alpha\text{-(3-indolyl)-propionic acid}$ is as large as 71° . The maximum in the melting point curve corresponding to the molecular compound in this case was for the greater part overlapped by the branch of the higher melting component (Fig. 15). However, X-ray powder photographs revealed the existence of a molecular compound between $(-)\text{-}\alpha\text{-(1-naphthyl)-propionic acid}$ and $(+)\text{-}\alpha\text{-(3-indolyl)-propionic acid}$ (5). The melting point curve for the system containing acids with the same direction of rotation can be seen in Fig. 16.

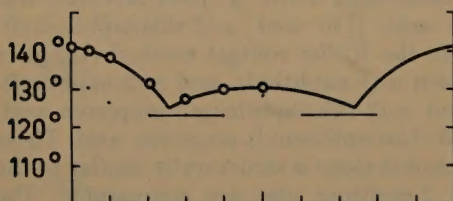
Bearing in mind the structural similarities between $\alpha\text{-(1-naphthyl)-propionic acid}$ (II) and $\alpha\text{-(4-methyl-1-naphthyl)-propionic acid}$ (III) and the great racemate formation tendency of the acids (Figs. 2 and 3), a quasi-racemate could be expected between these two acids. A pronounced quasi-racemic compound was also formed between $(+)\text{-}\alpha\text{-(4-methyl-1-naphthyl)-propionic acid}$ and $(-)\text{-}\alpha\text{-(1-naphthyl)-propionic acid}$ (Fig. 17). The acids with the same direction of rotation gave a simple eutectic diagram (Fig. 18). $(+)\text{-}\alpha\text{-(4-Methyl-1-naphthyl)-propionic acid}$ can consequently be represented by stereoformula III.



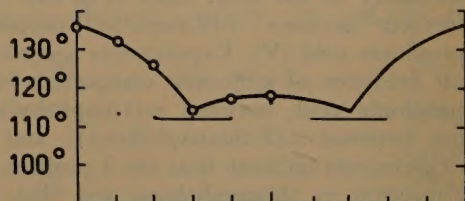
Mole-% (+)-acid
Fig. 2. (+)- and (-)- α -(1-naphthyl)-propionic acid (4).



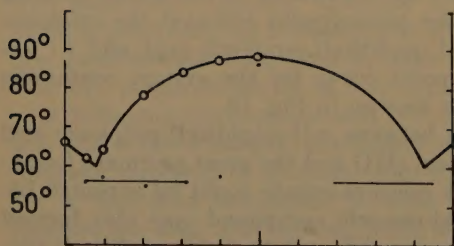
Mole-% (+)-acid
Fig. 3. (+)- and (-)- α -(4-methyl-1-naphthyl)-propionic acid (7).



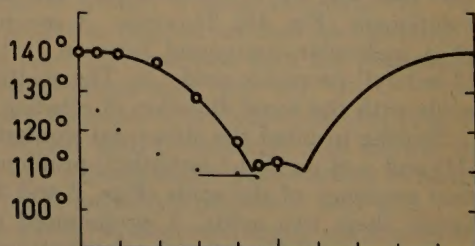
Mole-% (+)-acid
Fig. 4. (+)- and (-)- α -(2-naphthyl)-propionic acid.



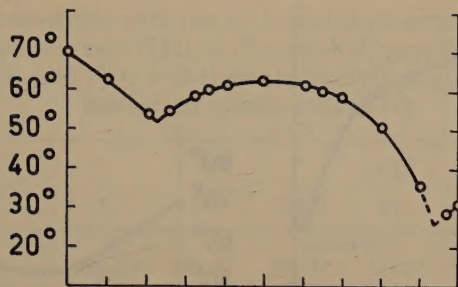
Mole-% (+)-acid
Fig. 5. (+)- and (-)- α -(2-thianaphthenyl)-propionic acid.



Mole-% (+)-acid
Fig. 6. (+)- and (-)- α -(3-thianaphthenyl)-propionic acid (4).

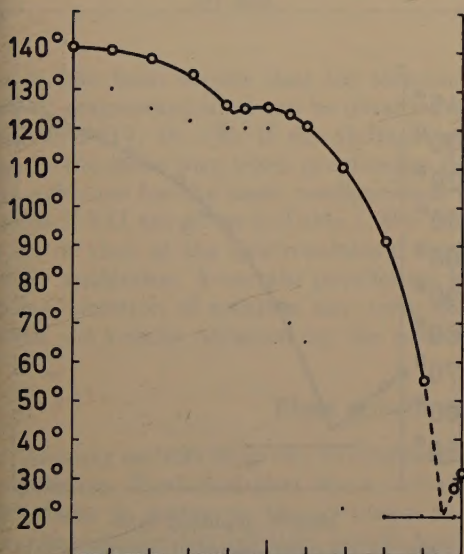


Mole-% (+)-acid
Fig. 7. (+)- and (-)- α -(3-indolyl)-propionic acid (5).



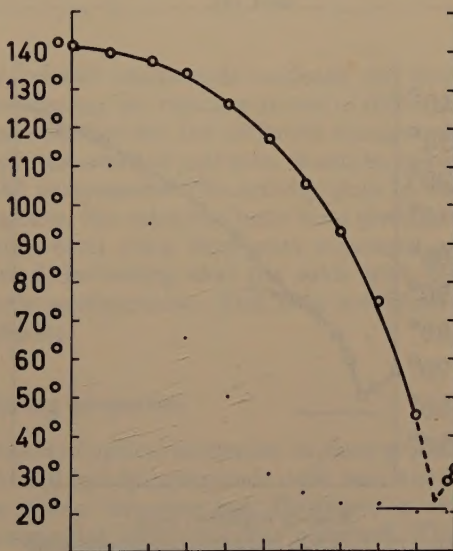
Mole-% hydratropic acid

Fig. 8. $(-)\text{-}\alpha\text{-(1-naphthyl)-propionic acid}$ and $(+)\text{-hydratropic acid}$ (according to Fredga, ref. 2).



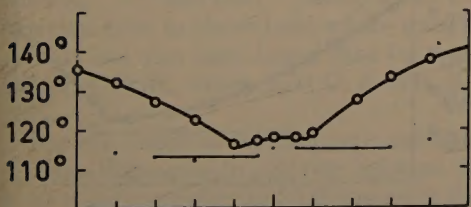
Mole-% hydratropic acid

Fig. 9. $(+)\text{-}\alpha\text{-(2-naphthyl)-propionic acid}$ and $(-)\text{-hydratropic acid}$.



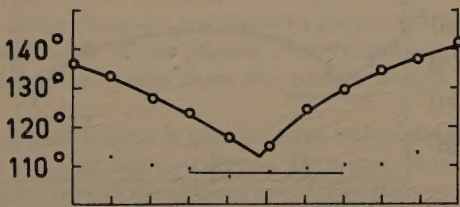
Mole-% hydratropic acid

Fig. 10. $(-)\text{-}\alpha\text{-(2-naphthyl)-propionic acid}$ and $(-)\text{-hydratropic acid}$.



Mole-% naphthyl acid

Fig. 11. $(-)\text{-}\alpha\text{-(2-naphthyl)-propionic acid}$ and $(+)\text{-}\alpha\text{-(2-thianaphthenyl)-propionic acid}$.



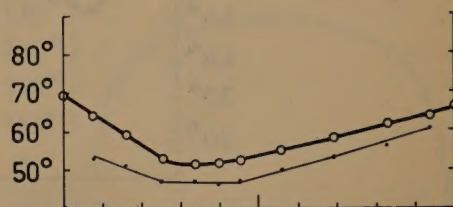
Mole-% naphthyl acid

Fig. 12. $(+)\text{-}\alpha\text{-(2-naphthyl)-propionic acid}$ and $(+)\text{-}\alpha\text{-(2-thianaphthenyl)-propionic acid}$.



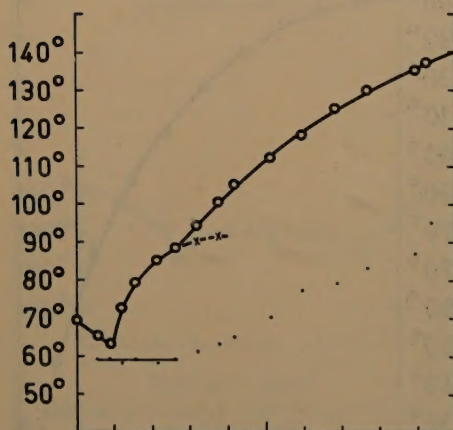
Mole-% thianaphthenyl acid

Fig. 13. (–)-α-(3-thianaphthenyl)-propionic acid and (+)-α-(1-naphthyl)-propionic acid (4).



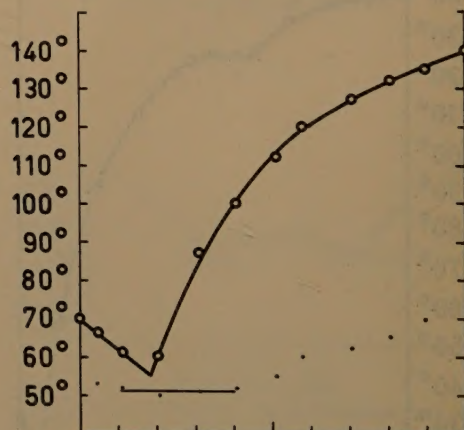
Mole-% thianaphthenyl acid

Fig. 14. (–)-α-(3-thianaphthenyl)-propionic acid and (–)-α-(1-naphthyl)-propionic acid (4).



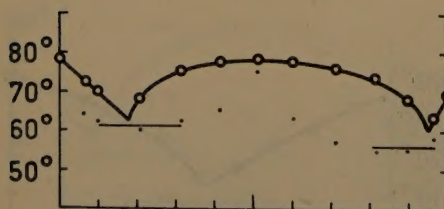
Mole-% naphthyl acid

Fig. 15. (–)-α-(1-naphthyl)-propionic acid and (+)-α-(3-indolyl)-propionic acid (5).



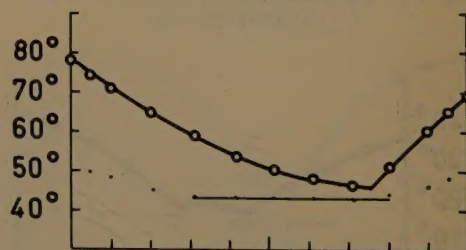
Mole-% naphthyl acid

Fig. 16. (+)-α-(1-naphthyl)-propionic acid and (+)-α-(3-indolyl)-propionic acid (5).



Mole-% naphthyl acid

Fig. 17. (–)-α-(1-naphthyl)-propionic acid and (+)-α-(4-methyl-1-naphthyl)-propionic acid (7).



Mole-% naphthyl acid

Fig. 18. (+)-α-(1-naphthyl)-propionic acid and (+)-α-(4-methyl-1-naphthyl)-propionic acid (7).

Table 1. $[M]_D^{25}$ for hydratropic acid (I), α -(1-naphthyl)-propionic acid (II), α -(4-methyl-1-naphthyl)-propionic acid (III), α -(2-naphthyl)-propionic acid (IV), α -(2-thianaphthenyl)-propionic acid (V), α -(3-thianaphthenyl)-propionic acid (VI) and α -(3-indolyl)-propionic acid (VII).

Solvent	$[M]_D^{25}$						
	I	II	III	IV	V	VI	VII
Benzene	138.8°	361.5°	331.7°	154.6°	28.7°	226.1°	200.9°
2,2,4-Trimethylpentane . .	125.3°	342.4°	336.7°	164°	93°	215°	184°
Acetic acid.	131.1°	304.8°	297.0°	155.0°	72.6°	206.5°	181.8°
Acetone	143.3°	292.1°	277.5°	196.4°	106.9°	190.2°	174.6°
Chloroform.	113.1°	268.2°	247.7°	142.5°	45.0°	171.2°	157.8°
Ethanol	118.8°	240.9°	233.5°	137.7°	64.4°	165.5°	150.2°
Water (ion)	8.7°	69.9°	47.4°	-34.8°	-54.3°	2.3°	-32.4°

It has been shown that for structurally related compounds evidence for their steric connection often can be obtained by comparing the rotatory power in different solvents (17, 18, 19). If the shifts in optical rotation for the different compounds go in the same way when progressing through the series of solvents, it can be taken as evidence for the same configuration of the compounds. The activity data of the acids I-VII are given in Table 1. For convenience, the rotations have been given the sign of that of the dextrorotatory compounds even when they were measured on their antipodes. A certain parallelism is found, indicating that the acids with the same direction of rotation also have the same configuration. This is in accordance with the results obtained by the other methods.

Plant growth regulating properties

As early as 1937 Kögl (20, 21) studied the effect of optical isomerism in plant growth regulators. He found that the (+)-form of α -(3-indolyl)-propionic acid was about 30 times as active as the (-)-form in the *Avena curvature test*. However, in the *Avena cylinder test* (straight growth test) no essential difference was observed. This fact was ascribed to different physiological transport of the antipodes in the *curvature test*. It was furthermore stated that the two enantiomers should have the same activity if they acted directly upon the cells of the growth zone (21). Later investigations in this field, among others by Fredga, Matell (8, 22, 23) and Åberg (24), have shown that the (+)- and the (-)-antipode of nearly all the investigated acids have different activity even in those tests where transport differences do not seem to play a major role. It has also been found that for a series of acids of the general structure Ar-X-CH(R)-COOH , where X is O, S, NH or CH_2 , the D-forms have the greater activity (25).

For the series of arylpropionic acids investigated there is also in most cases a significant difference in activity between the (+)- and the (-)-forms. However, there seem to be some essential differences in the plant growth regulating properties between the aryloxy and the aryl carboxylic acids. In the series of aryloxy carboxylic acids only the D-forms were auxins while the corresponding L-forms could be inactive or more or less active anti-auxins. In some cases both the D- and the L-forms were

Table 2. Plant growth regulating activity and configuration of the investigated aryl carboxylic acids.

Acid	Avena cyl. test		Wheat root test		Flax root test	Type of activity		Configuration
	C_{inhib} 20 %	C_{stim} 40 %	C_{stim} 20 %	C_{inhib} 50 %	C_{inhib} 50 %	auxin	anti-auxin	
Phenylacetic		3×10^{-5}		1×10^{-5}	3×10^{-6}	++		
(+)- α -Phenylpropionic		^a		8×10^{-6}	3×10^{-6}	++		D
(-)- „	1.5×10^{-5}		^b	1×10^{-4}	7×10^{-5}		++	L
1-Naphthylacetic . . .		5×10^{-7}		1×10^{-7}	7×10^{-8}	+++		
(+)- α -(1-Naphthyl)-propionic		1×10^{-6}		3×10^{-7}	1.5×10^{-7}	+++		D
(-)- „		1×10^{-4}		1.5×10^{-6}	1.0×10^{-7}	++		L
4-Methyl-1-naphthylacetic		3×10^{-6}		6×10^{-7}	5×10^{-7}	++		
(+)- α -(4-Methyl-1-naphthyl)-propionic	5×10^{-7}	(+)		5×10^{-5}	4×10^{-6}	(+)	(+)	D
(-)- „	5×10^{-7}	(+)	1×10^{-6}	5×10^{-5}	1×10^{-6}	(+)	+	L
2-Naphthylacetic	7×10^{-7}	+	3×10^{-7}	4×10^{-6}	2×10^{-7}	+	+	
(+)- α -(2-Naphthyl)-propionic	1.5×10^{-6}	(+)	5×10^{-8}	5×10^{-5}	5×10^{-6}	(+)	++	D
(-)- „	1.5×10^{-6}	(+)	3×10^{-7}	5×10^{-5}	8×10^{-7}	(+)	++	L
2-Thianaphthenylacetic	1×10^{-4}			5×10^{-5}	1×10^{-4}		++	
(+)- α -(2-Thianaphthenyl)-propionic . .	2×10^{-6}	(+)	1.5×10^{-7}	$> 3 \times 10^{-5}$	8×10^{-6}	(+)?	+++	D
(-)- „	5×10^{-6}		1×10^{-7}	3×10^{-5}	1×10^{-5}		+++	L
3-Thianaphthenylacetic	3×10^{-8}	2×10^{-5}		1.5×10^{-7}	1×10^{-7}	++	+	
(+)- α -(3-Thianaphthenyl)-propionic . .		2×10^{-7}		4×10^{-8}	5×10^{-8}	+++		D
(-)- „	1×10^{-6}	4×10^{-5}		1×10^{-6}	1.5×10^{-7}	++	(+)	L
3-Indolylacetic		1.5×10^{-7}		2×10^{-8}	7×10^{-9}	+++		
(+)- α -(3-Indolyl)-propionic		6×10^{-8}		2×10^{-8}	2×10^{-8}	+++		D
(-)- „		6×10^{-8}		1×10^{-7}	1×10^{-8}	+++		L

^a 20 % at 1×10^{-4} . ^b 15 % at 3×10^{-5} .

anti-auxins. The D-forms were more active than the corresponding L-forms in all the tests tried, though with a less pronounced difference between the antipodes for acids with low activity.

The plant growth regulating properties of the aryl carboxylic acids reported in this paper have been investigated by Professor B. Åberg who has kindly placed some of his unpublished results at the author's disposal. The biological activity was studied by means of the *Avena cylinder test*, the *wheat root test* and the *flax root test* (for short descriptions, see ref. 26). The results are found in Table 2. The auxin activity in the *Avena cylinder test* is illustrated by the lowest molar concentration (C_{stim}) causing a growth about 40 % higher than that of the control cylinders. C_{inhib} in the same test refers to the lowest molar concentration causing about 20 % inhibition

of the growth. In some cases the inhibition caused by relatively low concentrations tends to change over into a stimulation at higher concentrations (cp. 26). When the growth does not reach the control level this phenomenon is indicated by the sign (+) in the C_{stim} column, when the control level is reached it is indicated by +, and when the control level is greatly exceeded a concentration value as defined above for C_{stim} is given.

In the *wheat root test* there is often a growth stimulation at relatively low concentrations (antiauxin activity). This is illustrated in the table by the lowest molar concentration (C_{stim}) causing a growth about 20 % higher than that of control roots. At sufficiently high concentrations there is always a growth inhibition (auxin activity or toxic effects) which is characterized by the molar concentration depressing the growth to 50 % (C_{inhib}). For the *flax root test* only the inhibition effects are given (in the same way as for the wheat roots).

As can be seen in Table 2 for some of the acids the D- as well as the L-forms can be auxins. The enantiomers of α -(3-indolyl)-propionic acid have higher activity in the *Avena cylinder test* than 3-indolylacetic acid, and in this test no essential difference was observed between the antipodes. α -(1-Naphthyl)- and α -(3-thianaphthenyl)-propionic acid are acids for which both the antipodes are auxins. As mentioned above the D-forms of the aryloxy carboxylic acids were more active than the corresponding L-forms in all the tests tried. For the investigated aryl carboxylic acids, however, in some cases the L-forms are slightly more active than the corresponding D-forms in the *flax root test*. This type of inversion has been found for α -(1-naphthyl)-, α -(4-methyl-1-naphthyl)-, α -(2-naphthyl)- and α -(3-indolyl)-propionic acid.

Experimental

Melting point diagrams

Weighed amounts of the components were dissolved in a few drops of pure acetone. The solvent was evaporated and the residue carefully powdered and dried in a desiccator. The melting points were determined in a Kofler hot stage microscope (15). All preparations, except those containing α -(3-indolyl)-propionic acid, were stable for three subsequent meltings. A small change in melting point could be observed for α -(3-indolyl)-propionic acid when the melting-crystallization process was repeated. The reproducibility of the melting points was better than 1°.

Optical activity in different solvents

Weighed amounts of the acids were dissolved in the different solvents, made up to a volume of 10.00 ml and the rotatory power was determined. In the case of water the acids were neutralized with dilute sodium hydroxide to pH 7. The results are given in Table 1.

X-ray powder photographs

The photographs were taken in a focusing camera of the Guinier type with Cu-K α radiation. The preparations were obtained in the same way as described for the melting point diagrams.

SUMMARY

The configuration of α -(2-naphthyl)-propionic acid has been established. The D-configuration of (+)-hydratropic acid has been proved. The steric relations of the following aryl carboxylic acids have been reviewed: α -phenyl-, α -(1-naphthyl)-, α -(4-methyl-1-naphthyl)-, α -(2-naphthyl)-, α -(2-thianaphthenyl)-, α -(3-thianaphthenyl)- and α -(3-indolyl)-propionic acid as well as α -methyl- γ -phenylbutyric acid. The acids with the same direction of rotation also have the same configuration. The dextrorotatory acids are related to the D-glyceraldehyde system.

Preliminary results of the plant growth regulating properties of the enantiomers of the propionic acids are reported.

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Department of Organic Chemistry, Chemical Institute, University of Uppsala, December 1957.

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Electronic and steric effects on the ultraviolet and infrared spectra of some organic selenium compounds

By GÖRAN BERGSON

With 14 figures in the text

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Introduction

In organic chemistry it has become customary to account for different phenomena in terms of various "effects". Concepts found in the literature are steric, inductive, mesomeric, conjugative, hyperconjugative effect etc. A dilemma in present day theoretical organic chemistry is that it is not always easy to set up a generally valid definition of the different concepts, and furthermore that they are not always separable. It is even questionable if the separation of steric effects from electronic effects always has a sound theoretical background. The so-called electronic effects have appeared in a curious manner. From the theoretical point of view it has always been evident that a localization of electrons to valence bonds is only a rough approximation, which for compounds such as saturated hydrocarbons is fairly good, but for compounds like butadiene or benzene is rather bad. But because organic chemists want to adhere to the concept of localized valence bonds, when this concept operates badly, new "effects" are introduced. Nevertheless, such effects are often useful in systematizing observed facts. In this paper use will be made of the electronic effects known as the inductive effect and the hyperconjugative effect. But the author wants to point out that the study of these effects on UV and IR spectra of selenium compounds is only an empirical one with the view to but the properties observed in

correlation with conclusions drawn from other fields of organic chemistry. The steric effect treated in this paper is an effect due to changes in conformation of the diselenide group. A recent molecular orbital calculation of the energetics of the disulphide bond as a function of the conformation (valid also for diselenides, but numerically treated only for disulphides) suggests that there might exist a sound theoretical background to this kind of steric effect (1).

Why is it appropriate to study electronic and steric effects on selenium compounds? The answer is, that in connection with studies on sulphur and selenium compounds at this institute, it soon became evident that diselenides were excellent as model substances for a study in the ultraviolet, because the first absorption band was wholly within the near ultraviolet (2). Furthermore, due to the size of the selenium atom, steric interaction between the substituents can often be neglected in diselenides, which permits a study of pure electronic effects. Therefore, empirical correlations between observed absorption spectra and the above mentioned effects were easily found (2, 3). As has been exemplified in the studies by Schotte (4, 5, 6, 7) and the present author (2, 3) this contributed to the interpretation of the spectra of disulphides.

This paper summarizes the data obtained during the last three years work at this institute. A few of the UV spectra of the diselenides have been published before (2, 3), but no IR spectra have appeared in print.

1. Ultraviolet spectra

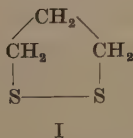
(a) *Experimental and previous measurements*

The UV spectra have been recorded with a Beckman model DU spectrophotometer in the region 2200–4000 Å, and in the visible region with a Beckman model B instrument. 96 % ethyl alcohol was used as a solvent when no specification is made. Solvent effects are small for the substances treated in this paper.

In recent years systematic studies of the UV spectra of aromatic selenium compounds have been made by Chierici and Passerini. They have studied some phenyl selenides (8, 9) and phenyl alkyl selenides (10) and the steric effect in the UV spectrum of mesityl methyl selenide (11). Furthermore a large number of sym.-diaryl diselenides have been studied and their spectra interpreted (12). Investigations of phenyl seleninic acids must also be mentioned (13). In 1955 Kiss and Muth (14) reported the spectra of *o,o'*-dicarboxylic acids of diphenyl selenide and diphenyl diselenide. Due to the interaction between the selenium atom and the aromatic nucleus, the interpretation of the UV spectra of aromatic selenium compounds must take a quite different course than that for aliphatic compounds. Nevertheless the aromatic compounds are of great theoretical interest. Schotte's exhaustive discussion of the spectra of the isomeric dithiodibenzoic acids and their methyl esters must be mentioned in this connection (6). Bergson has found that Schotte's interpretations are also valid for those of the corresponding selenium compounds studied (15). The close analogy between the aromatic sulphur and selenium compounds is also well established by the work of Chierici, Passerini, Kiss and Muth mentioned above. Of great importance for the following discussion are the findings of Margolis and Pittman (16). They have studied the UV absorption of di-*iso*-propyl diselenide, di-*sec*-butyl diselenide and bischloromethyl diselenide. Their data confirm the relationships found by the present author, which will be discussed in more detail below.

(b) General theory

Pauling (17) predicted that in a compound with two selenium atoms joined to each other, the dihedral angle would tend to be about 90° , due to the interaction between the $4p\pi$ lone pairs of electrons. This has been confirmed by experiment (18). The behaviour of the diselenides must therefore be expected to be analogous to that of the disulphides (17, 18) for which more data are available. It is known that for disulphides a decrease in dihedral angle, φ , causes a red shift (from $2500 \text{ \AA}$ for $\varphi = 90^\circ$ to $3300 \text{ \AA}$ for $\varphi = 27^\circ$). It has been supposed that this shift is due to the $3p\pi$ electrons (19), but the first explanation was given in a recent paper by Bergson (1). Using a simple molecular orbital theory, conformation analysis was performed for the ground and the excited state of the disulphide molecule. The results of the investigation is given again in Fig. 1. The decrease in excitation energy (red shift) with decreasing dihedral angle is evident. For 1,2-dithiolane (I) the absorption peak was calculated to be at $3250 \text{ \AA}$ compared with the experimental value, $3300 \text{ \AA}$. Because of the lack of information concerning the magnitude of the torsion barrier in the ground state of a diselenide, the theory unfortunately cannot be tested for diselenides at the present time. It is evident, however, that diselenides behave in analogy with the disulphides. The only thing which is different is the γ -function and the numerical coefficients in Fig. 1. ($\gamma = \beta - \alpha S$, where α is the Coulomb integral, β the exchange integral and S the overlap integral. The numerical coefficients are functions of S only. One further comment to the theory of this steric effect is necessary. The conformation analysis and therefore the interpretation of the spectra could scarcely have been performed without quantum mechanics. Without it the erroneous conclusion might have been made that in the ground state the interaction between the $p\pi$ electrons was Coulombic repulsion between the charge clouds. The γ -function, however, represents a potential energy in the electrostatic field from the nuclei (20). Furthermore, as is explained in ref. 1, the S-S bond is strongly weakened in the first excited state because the electron occupies an antibonding σ -orbital. As seen from Fig. 1 this weakening decreases with decreasing dihedral angle due to the $3p\pi$ interaction (contrary to the behaviour in the ground state). In the ionized state, however, the S-S bond must be approximately as strong as in the ground state if $\varphi = 90^\circ$, and even stronger if $\varphi = 0^\circ$ (by an amount of about $0.624|\gamma| = 15 \text{ kcal/mol}$). This explains the so called "additional bond" spoken of in the ionized cystine molecule (21). Fig. 2 *qualitatively* represents these matters.



The *inductive effect* of a substituent causes a change in effective nuclear charge of the chromophore (the Se-Se group). Increased nuclear charge causes an increased excitation energy. Thus an electron attracting substituent (a carboxylic group or a halogen atom) increases the excitation energy (hypsochromic effect, blue shift). This is at least true for $N \rightarrow R$ transitions in molecules, but exceptions at other types of transitions are not lacking (22).

It is well known that butadiene absorbs light at longer wave lengths than ethylene. This decrease in excitation energy is due to the delocalization of the $2p\pi$ electrons.

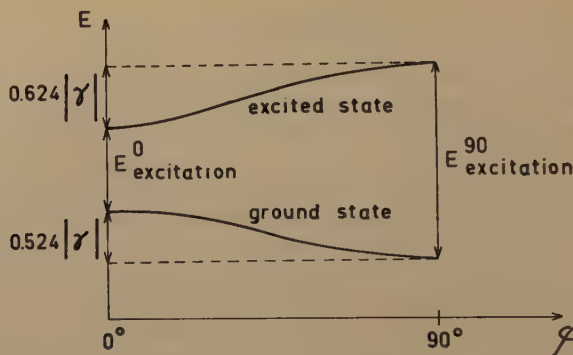


Fig. 1. The energetics of the disulphide bond as a function of the dihedral angle. (From Bergson, *Arkiv Kemi* 12, 233 (1958).)

Such a delocalization always leads to an approach of the ground and the excited state to each other. σ -electrons are also delocalized but to a lesser extent than π -electrons. Delocalization of σ -electrons is often termed *hyperconjugation* in contrast to conjugation which means delocalization of π -electrons. But as Mulliken (23) has pointed out, the difference in the delocalization of the electrons in a methyl group and in e.g. a triple bond is quantitative rather than qualitative in nature. The σ -electrons which are by far the most delocalized are the C-H σ -electrons, and hyperconjugation is therefore only considered for C-H bonds. The order of hyperconjugation for alkyl groups is therefore (24): $\text{CH}_3 > \text{CH}_3\text{CH}_2 > (\text{CH}_3)_2\text{CH} > (\text{CH}_3)_3\text{C}$.

It is thus evident that hyperconjugation decreases the excitation energy in a molecule (bathochromic effect, red shift). The spectra of methyl substituted ethylenes have been treated in Coulson's textbook (20). The red shift on methyl substitution in ethylene is in part due to the inductive effect of the methyl groups, but Coulson shows that a large part of the red shift must be due to hyperconjugation between the methyl groups and the double bond. Other experimental evidence for hyperconjugation

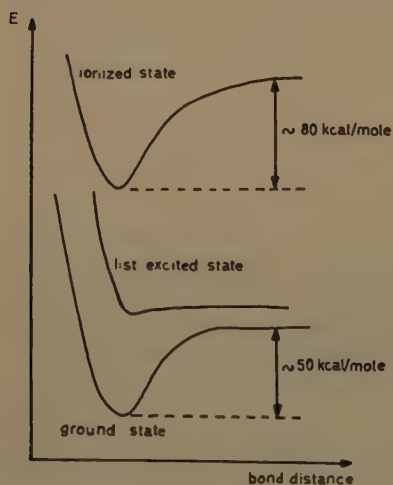


Fig. 2. The energetics of the disulphide or diselenide bond as a function of the bond distance. $\phi = 0^\circ$. Not drawn to scale.

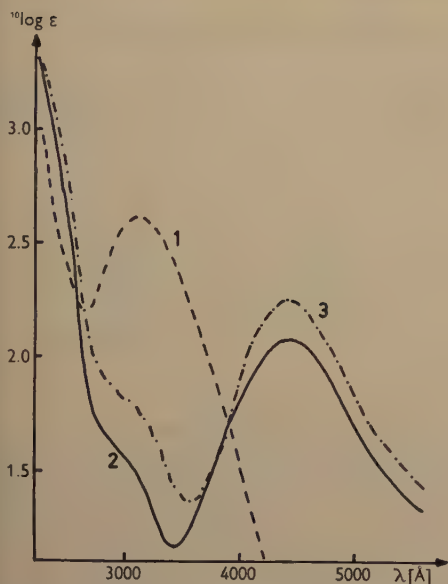


Fig. 3. Ultraviolet spectrum of (1) diethyl-diselenide, (2) 4,4-bishydroxymethyl-1,2-diselenolane and (3) 6-selenoctic acid. Cf. ref. 28 and 29 for the preparation of these compounds.

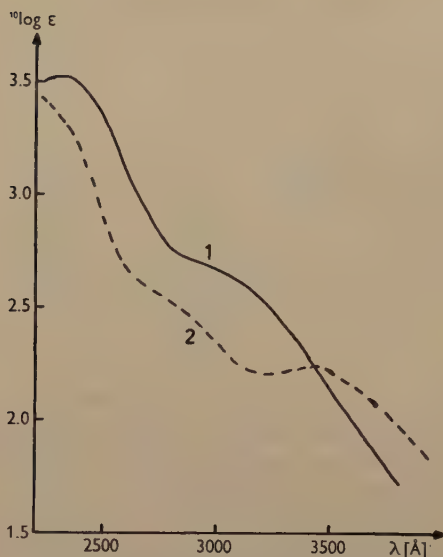


Fig. 4. Ultraviolet spectrum of (2) 1,2-diselenane-3,6-dicarboxylic acid and (1) diselenium- α -di-propionic acid. Cf. ref. 27 for the preparation of these compounds.

tion is found in Baker's textbook (24): Thanks to the ability of sulphur and selenium to expand their valence shells to a decet, hyperconjugation might be possible in sulphur and selenium compounds. Here the sulphur and selenium atom corresponds to the $C=C$ group in e.g. ethylene. For disulphides and diselenides this effect was pointed out by the present author (2, 3) and the results are discussed in detail below. In valence bond language this sort of hyperconjugation may be pictured as: $H-C-S \leftrightarrow H^+C=S^-$.

Hyperconjugation in sulphonium compounds has been used to explain their difference in reactivity as compared with the corresponding quaternary ammonium derivatives in which hyperconjugation is impossible because of the inability of nitrogen to expand its octet. The hyperconjugation in sulphonium compounds is represented as: $H-C-S^+R_2 \leftrightarrow H^+C=SR_2$ (24).

(c) UV spectra of diselenides

The *steric effect* is the largest of the effects studied, and it is most pronounced when the selenium-selenium group is situated in a five-membered ring as exemplified in Fig. 3, where the spectrum of diethyl diselenide (II) is shown together with that of 4,4-bishydroxymethyl-1,2-diselenolane (III) and 6-selenoctic acid (IV). The unsubstituted 1,2-diselenolane (V) has a spectrum very similar to that of the other 1,2-diselenolane derivatives, but because of experimental difficulties the molar extinction coefficient could not be determined (see ref. 25 for a plot of the optical density as a function of the wavelength and further information about this substance). The

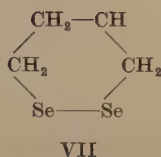
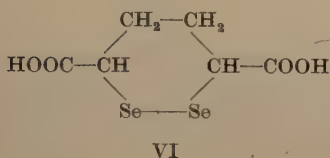
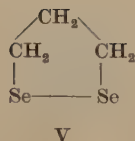
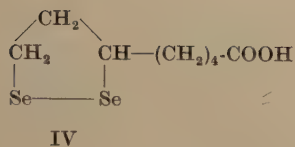
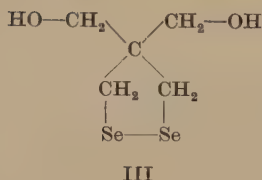
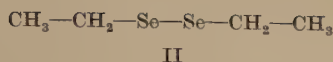
Table 1. Ultraviolet absorption measurements on cyclic diselenides.

Compound	Ring size	$\lambda_{\max}$ Å	$10 \log \epsilon_{\max}$	E_{excit} kcal/mol	$\Delta E_{\text{excit.}}$ kcal/mol
1,2-Diselenolane (V)	5	4440 ^a	—	64.4	27.3
4,4-bishydroxymethyl-1,2-diselenolane (III) . .	5	4430	2.09	64.5	27.2
6-selenoctic acid (IV)	5	4420	2.25	64.7	27.0
1,2-diselenane (VII)	6	3650 ^a	—	78.3	13.4
(+)-1,2-diselenane-3,6-dicarboxylic acid (VI) .	6	3410	2.25	83.8	13.7
rac.-1,2-diselenane-3,6-dicarboxylic acid (VI) .	6	3430	2.31	83.3	14.2
meso-1,2-diselenane-3,6-dicarboxylic acid (VI) .	6	3450	2.35	82.8	14.7

^a Measured in chloroform solution.

red shift observed when going from diethyl diselenide to the 1,2-diselenolane derivatives, corresponding to a change in dihedral angle from about 90° to a much smaller one, is in good agreement with the theoretical predictions made above. In a six-membered cyclic diselenide the steric effect should not be so pronounced since the dihedral angle does not deviate so much from 90° in such a ring. Foss (26) has determined the dihedral angle in 1,2-diselenacyclohexane-3,6-dicarboxylic acid (VI) to 56°, and the UV peak for this acid is at 3450 Å as can be seen from Fig. 4, where comparison is made with diselenium- α -di-propionic acid. In (VI) the carboxylic groups cause a hypsochromic shift, and the absorption maximum for the unsubstituted six-membered ring (VII) 3650 Å is a better one for comparison. Thus for open, six-membered and five-membered diselenides we have to compare the values 3120, 3650 and 4440 Å respectively. All the measurements on cyclic diselenides are summarized in Table 1.

The solutions of 1,2-diselenolane and 1,2-diselenane were obtained by depolymerization of the polymers according to the method of Bergson and Claeson (25). Chloroform was used as a solvent because these solutions appeared to be somewhat more stable than the alcoholic solutions. In column 4 the logarithm of the molar extinction coefficient is given, and it is remarkably constant which indicates that it is really the same transition which is studied in all the molecules. Column 5 gives the excitation energy in kcal/mol. These numbers are calculated from the values of $\lambda_{\max}$ by means of the relation $E_{\text{excitation}} = hc/\lambda_{\max}$. Numerically this relation is $E_{\text{excitation}} = 2.86 \times 10^5/\lambda_{\max}$ if λ is measured in Å and $E_{\text{excitation}}$ in kcal/mol. $\Delta E_{\text{excitation}}$ in column 6 measures the difference in excitation energy between the cyclic diselenide and the corresponding linear one, i.e. a linear diselenide with the same substituents at the α -carbon atom as the cyclic one and thus with the same "electronic effects". (This is not strictly true for 6-selenoctic acid, which is compared with a diselenide with two hydrogens at each α -carbon atom.) In the case of the six-membered carboxylic acids comparison must be made with diselenium- α -di-propionic acid which shows only an inflection at 2930 Å. This of course introduces some uncertainties. In spite of this we can certainly stress the close similarities in the ΔE values for these compounds (13.7–14.7) and that for 1,2-diselenane (13.4), which indicates that the "electronic effects" are about the same in the linear- and six-membered diselenides. If such conclusions can also be made for 5-membered rings cannot be answered from the data presented. Recently Claeson (34) has found that this cannot be done for 5-membered *disulphides*. The values of $\Delta E_{\text{excitation}}$ are therefore a direct measure of the steric



effect in the UV spectra of the diselenides, but from what has been said in part 1b of this paper and the discussion below, it would be a serious mistake to correlate these figures with the steric effect in the ground state of the molecules (the steric strain).

As mentioned above, the present author has given a simple theory which explains the steric effects on the UV spectra. Fig. 1 of this paper summarizes the theory. The mathematical expressions for the functions pictured in Fig. 1, i.e. the torsion energy in the ground and excited states are as follows; where E_{torsion}^0 refers to the ground state and E_{torsion}^x to the excited state. S is the overlap integral for the $p\pi$ orbitals and γ is equal to $\beta - \alpha S$, where β is the exchange integral and α the Coulomb integral.

$$E_{\text{torsion}}^0(\varphi) = \frac{-4S \cos^2 \varphi}{1 - S^2 \cos^2 \varphi} \gamma, \quad (\text{a})$$

$$E_{\text{torsion}}^x(\varphi) = \frac{(1 - 3S \cos \varphi) \cos \varphi}{1 - S^2 \cos^2 \varphi} \gamma. \quad (\text{b})$$

These relations have been derived in an unpublished work by the present author. Since the excitation energy is the difference between the curves of Fig. 1, we obtain, from the relations (a) and (b) and by making use of the excitation energy for $\varphi = 90^\circ$, the theoretical expression for $\Delta E_{\text{excitation}}$ of Table 1.

$$\Delta E_{\text{excitation}}(\varphi) = -\frac{\gamma \cos \varphi}{1 - S \cos \varphi}. \quad (\text{c})$$

Both that part of the steric strain which is due to torsion about the sulphur-sulphur bond (E_{torsion}^0) and the red shift in spectra ($\Delta E_{\text{excitation}}$) are proportional to γ , but it must be noted that the torsion energy also is proportional to the overlap integral S .

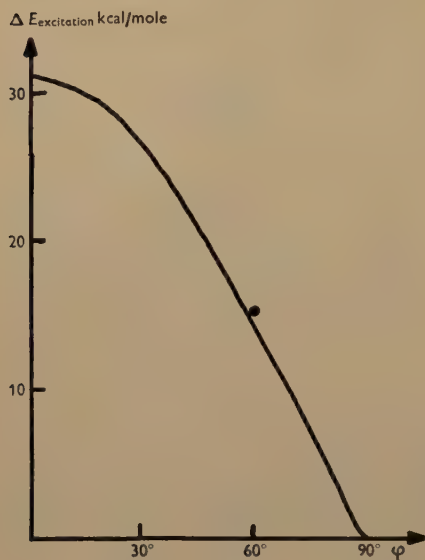


Fig. 5. The red shift in UV spectrum as a function of the dihedral angle for unsubstituted disulphides. The curve is calculated from equ. (c). The dot represents the experimental value for a six-membered cyclic disulphide.

That means that even if the strain is zero ($S = 0$ which is often supposed in approximate work) the red shift is $\beta \cos \varphi$. The rather complex relation between the torsion energy and the red shift is perhaps best illustrated if we combine equ. (a) and (c):

$$E_{\text{torsion}}^0(\varphi) = \frac{4S \cos \varphi}{1 + S \cos \varphi} \Delta E_{\text{excitation}} \quad (\text{d})$$

It is therefore necessary to know both S and φ in order to calculate the torsion energy from the red shift in UV spectra. The fact that $\Delta E_{\text{excitation}}$ for five-membered *disulphides* is the same as that for diselenides (27 kcal/mol) does not say that the strains in disulphides and diselenides are the same. It rather tells us that such is not the case, since the overlap integrals for sulphur and selenium are certainly not equal, and perhaps not even the dihedral angle φ . Unfortunately, the overlap integral for selenium atoms has not been calculated because of difficulties arising in connection with the non-integer effective quantum number occurring as an exponent in the Slater atomic wave functions. Furthermore it would be profitable to know the exact dihedral angle for a five-membered diselenide. It is perhaps of interest to see how the theory operates in the case of *disulphides*, where S and φ are known ($S = 0.129$; $\varphi = 27^\circ$ for a five-membered ring and 60° for a six-membered one (26)). From the experimental values for the five-membered ring γ is calculated in equ. (c), and the $\Delta E_{\text{excitation}}$ is plotted as a function of φ according to (c). This is done in Fig. 5 where the dot for the six-membered ring is also printed. It falls very close to the curve as theory predicts. The torsion barrier, i.e. the torsion energy for $\varphi = 0^\circ$, is obtained from (a) to be 14 kcal/mol. This should be compared with the value obtained from independent Raman-spectroscopic studies, 10–14 kcal/mol (30).

Only one thing further must be said about the spectra of cyclic diselenides. The small difference in the spectra of *rac.*- and (+)-1,2-diselenane-3,6-dicarboxylic acid

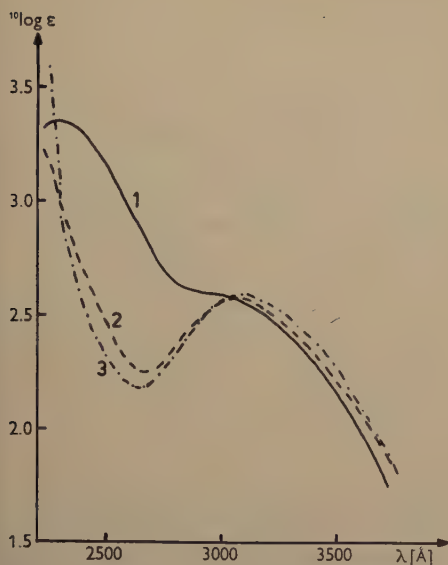


Fig. 6. UV absorption of $\text{HOOC}-(\text{CH}_2)_n-\text{Se}-(\text{CH}_2)_n-\text{COOH}$ with $n = 1, 2, 3$, for curves 1, 2, 3, respectively.

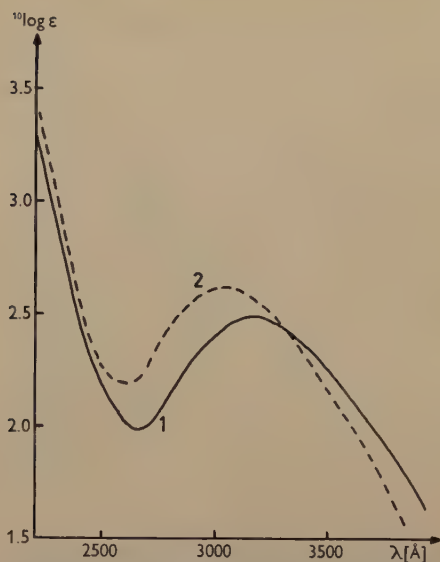


Fig. 7. UV absorption of (1) dimethyldiselenide and (2) di-iso-propyldiselenide.

cannot account for the deeper colour for the racemic acid compared with the optically active acid in the solid state as observed by Fredga (27). This difference is therefore certainly due to a solid state effect.

We now turn our attention to non-cyclic diselenides, in which we assume that the dihedral angle is constant and about 90° (perhaps somewhat larger due to a slight interaction between the substituents). This assumption is justified because of the size of the selenium atoms, which makes the interaction between the substituents negligible even in a compound such as di-*tert*-butyl diselenide (2). These facts allow for a study of *electronic effects* separately.

The *inductive effect* can be studied in the series of compounds represented by the formula (VIII). The hyperconjugation is here constant, because in all compounds there are two hydrogen atoms joined to the α -carbon atom (the carbon atom which is bound to selenium). When $n = 4$ the carboxylic group is so far from the chromophore (the Se-Se group) that its inductive effect is not detectable which is seen from the fact that diselenium- δ -di-*n*-valeric acid has absorption maximum at $3110 \text{ \AA}$, and di-*n*-butyl diselenide at $3120 \text{ \AA}$. This difference is insignificant. When the carboxylic group approaches the chromophore the maximum is displaced to shorter wavelengths as predicted by theory and as can be seen from Fig. 6.

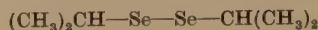
The *hyperconjugative effect* cannot unfortunately be studied separated from the inductive effect. If the inductive effect only was operative in the comparison between di-*tert*-butyldiselenide and di-*n*-butyldiselenide, the former must have the lowest excitation energy. This is not the case, and therefore the present author (2) suggested that some effect from the hydrogen atoms, which we might call hyperconjugation, is operative. The hyperconjugation must, according to theory cause a red shift, and if this effect is greater than the inductive effect of the methyl group, the observed



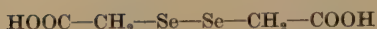
VIII



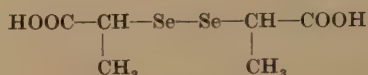
IX



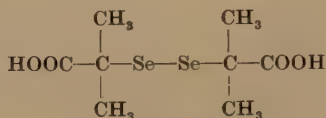
X



XI



XII



XIII

facts can be explained. The same thing can be seen from Fig. 7, where the spectrum of dimethyldiselenide (IX) and di-*iso*-propyldiselenide (X) with λ_{max} at 3160 and 3050 Å respectively are shown. The same general trends are also observed for disulphides (3). The absorption maximum for di-*tert*-butyldisulphide, however, lies at so low a wavelength that it is overlapped by the strong absorption at about 2000 Å, and cannot therefore be recorded. The spectra recorded by Margolis and Pittman (16) can be put exactly into the general scheme given here as is evident from Table 2. Changes in hyperconjugation is also found in the series represented by the formulae (XI), (XII) and (XIII). The absorption maximum for these acids is overlapped by the strong absorption at the vacuum-ultraviolet, and is only detectable as inflexions. But the general trend in this series is evident from Fig. 8. In Table 2 the results of the measurements are summarized.

(d) *Some concluding remarks*

We might now ask what use can be made of UV absorption measurements in organic chemistry. First of all, the more or less empirical rules for where to expect the position of the absorption maximum is a good guide in synthetic work. In the preparations of cyclic diselenides the present author made use of the expected red shift in order to control the success of the syntheses at an early stage. Secondly, the absorption intensity can be used to follow the progress of chemical reactions. This has been exemplified in the investigations of monomer-polymer equilibria studied by Bergson and Claeson (25) on 1,2-dithiolane and 1,2-diselenolane. But one had perhaps also hoped to be able to get a deeper insight of the molecules and their reactivities. Here, however, we have come to a serious dilemma. In organic-chemical reactions, it is only the ground state which is of importance. The steric and electronic effects on UV spectra measures only the difference between these effects on the ground and excited state. Thus in order to be able to calculate the effects of substituents etc. on the ground state from UV spectra, it is necessary to know these effects on the excited state *a priori*. But if we knew the effects on the excited state we would certainly

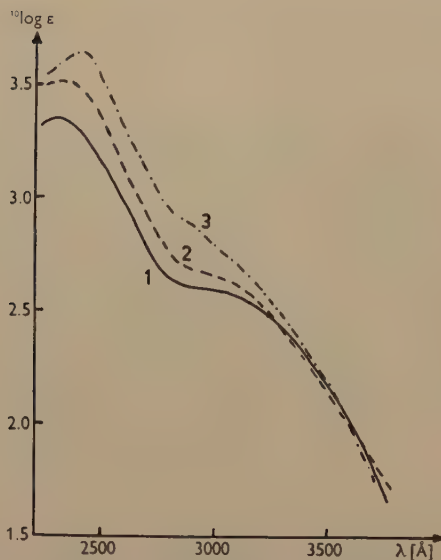


Fig. 8. UV absorption of (1) diselenium-diacetic acid, (2) diselenium- α -di-propionic acid, and (3) diselenium- α -di-*iso*-butyric acid.

know them still better in the ground state. Therefore, there is no high-road from UV spectra to chemical behaviour. Equ. (d) in this paper, however, shows that the road is passable even if great approximations must be made in the beginning. A similar treatment of the inductive and hyperconjugative effects is certainly much more complex. *We cannot therefore say whether e.g. the hyperconjugation has any importance for the ground state.* The IR investigations presented below might suggest that it has some importance.

Table 2. Ultraviolet absorption measurements on non-cyclic diselenides.

Compound	$\lambda_{\max}$ Å	$10 \log \epsilon_{\max}$	$E_{\text{excit.}}$ kcal/mol	$\Delta E_{\text{excit.}}$ kcal/mol
Di- <i>n</i> -butyldiselenide	3120	2.58	91.7	0.0
Diethyldiselenide	3115	2.62	91.8	(0.1)
Dimethyldiselenide	3160	2.49	90.5	1.2
Di- <i>iso</i> -propyldiselenide	3050	2.62	93.8	2.1
Di- <i>sec</i> -butyldiselenide	3050	2.64	93.8	2.1
Di- <i>tert</i> -butyldiselenide	2860	2.66	100.0	8.3
Bischloromethyldiselenide	3050	2.58	93.8	2.1
Diselenium-diacetic acid	2930 ^a	2.60	97.5	5.8
- β -dipropionic acid	3060	2.58	93.5	1.8
- γ -di- <i>n</i> -butyric acid	3080	2.58	92.8	1.1
- δ -di- <i>n</i> -valeric acid	3110	2.63	92.0	0.3
- α -dipropionic acid	2930 ^a	2.70	97.5	5.8
- α -di- <i>iso</i> -butyric acid	2870 ^a	2.90	99.7	8.0

^a Inflexion.

2. Infrared spectra

(a) Experimental

If the selenium compounds are very suitable for a study in the ultraviolet, because the absorption band is situated in an easily accessible wavelength region, their study in the infrared is all the more difficult. This is due to the fact that the vibrational absorption bands associated with selenium groups come at long wavelengths which require some special techniques. Calculations with approximate formulae such as (e) shows that C-Se stretchings are to be expected at 15–20 μ and Se-Se stretchings at about 40 μ . The present investigation has been carried out by means of a Perkin-Elmer model 21 recording spectrometer equipped with a caesium bromide prism.

The liquids were held in cells of 0.2 mm thickness with windows of polyethylene which is transparent in the whole region except at the very beginning at 15–15.5 μ . The solids were studied in emulsion in nujol, which is also transparent.

$$\nu = 1307 \sqrt{\frac{k}{\mu}} \quad \text{with} \quad k = 1.67 N \left(\frac{\chi_A \chi_B}{d^2} \right)^{\frac{3}{4}} + 0.30, \quad (e)$$

where μ is the reduced mass of the atom pair, N the bond order, χ_A and χ_B the electronegativities of the atoms A and B , and d the internuclear distance in Å. (See ref. 22 for further details about these formulae.)

(b) C-Se stretchings

In order to assign an IR peak to C-Se stretchings it is necessary to analyse a spectrum which is as simple as possible. The spectrum of di-*tert*-butyldiselenide has therefore been recorded in the whole wavelengths region (2–40 μ) as seen from Figs. 9 and 10. The peak at 3.50 μ is due to the C-H stretching and those at 6.90 and 7.36 μ are the asymmetric and symmetric C-H deformations respectively. The $(\text{CH}_3)_3\text{C}$ -skeletal deformation is found at 8.74 μ . The next peak, at 19.70 μ , is difficult to ascribe to anything else than a C-Se stretching. Some ill-defined peaks of low intensity are

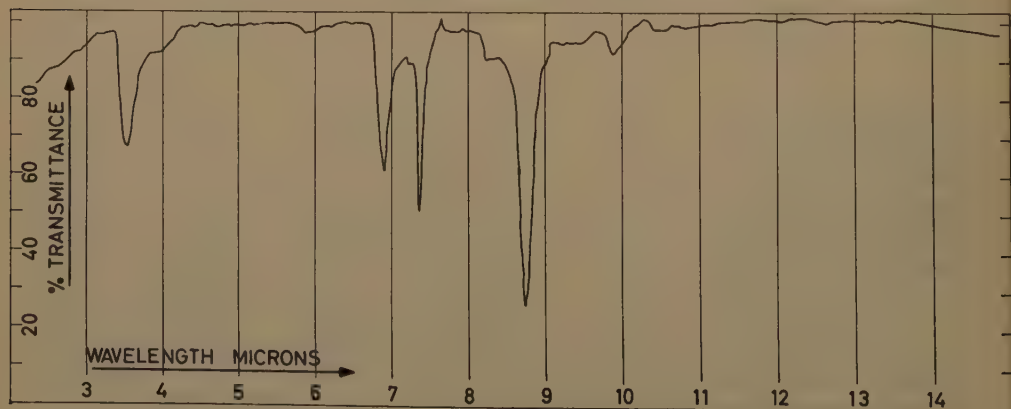


Fig. 9. Infrared spectrum of di-*tert*-butyl diselenide. 2–15 μ . NaCl prism.

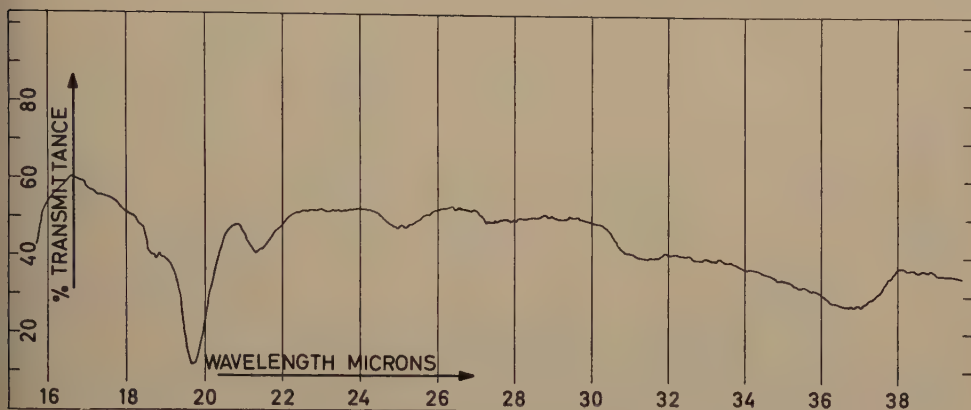


Fig. 10. Infrared spectrum of di-*tert*-butyl diselenide. 15–40 μ . CsBr prism.

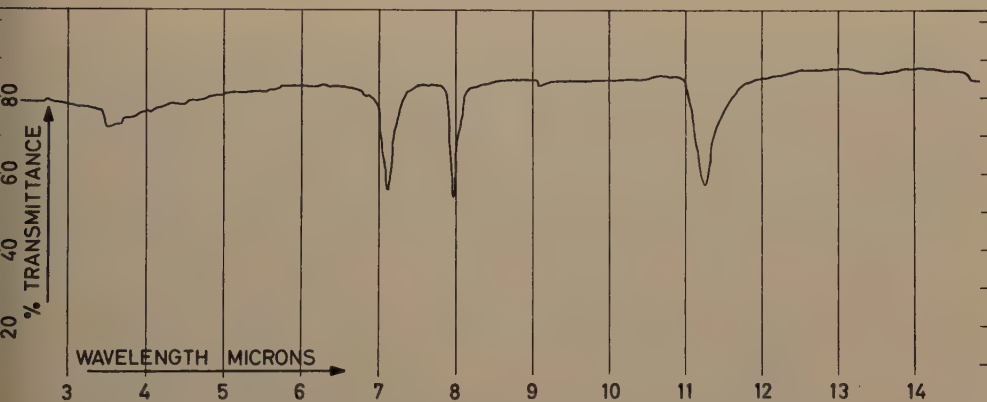


Fig. 11. Infrared spectrum of dimethyl diselenide. 2–15 μ . NaCl prim.

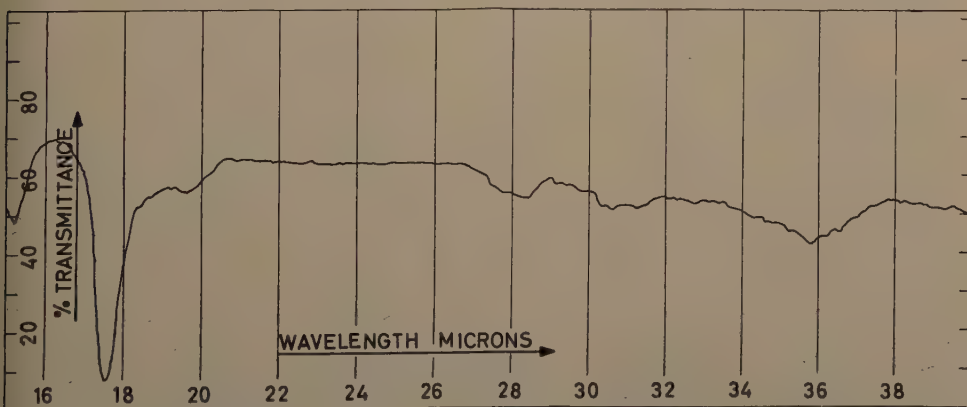


Fig. 12. Infrared spectrum of dimethyl diselenide. 15–40 μ . CsBr prism.

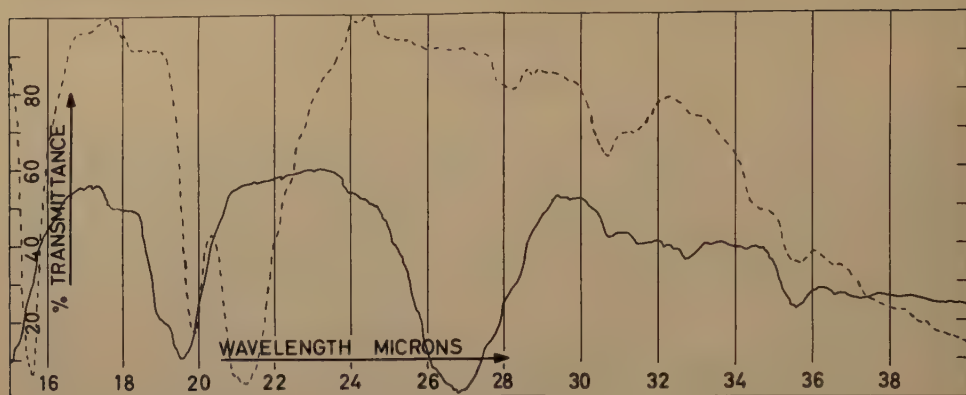
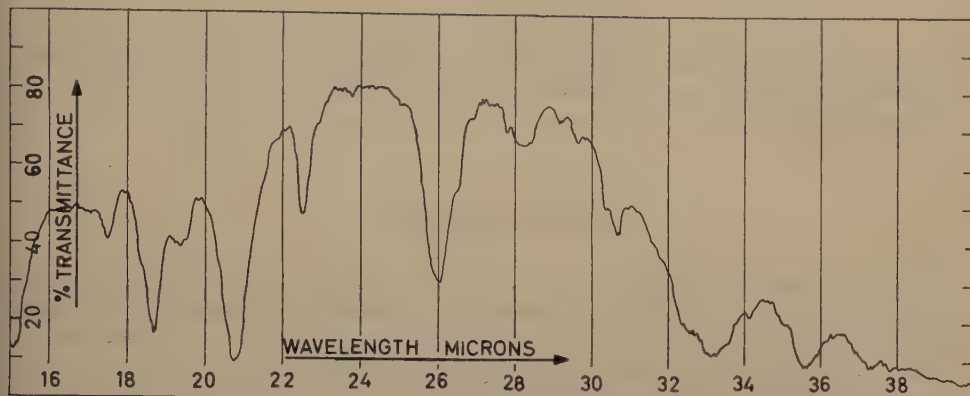


Fig. 13. Infrared spectrum of adipic acid and diseleniumdiacetic acid (dotted curve) 15–20 μ . CsBr prism.

observed at longer wavelengths. Dimethyldiselenide shows a similar spectrum (Figs. 11 and 12) but lacking of course the peak at 8.74 μ . But it shows an unassigned peak at 11.25 μ , and one at 17.55 μ . The latter is certainly due to the C–Se stretching. Diethyldiselenide, di-*n*-butyldiselenide and di-*iso*-propyldiselenide have peaks at 18.67, 18.60 and 19.35 μ respectively which are assigned to C–Se stretchings. Table 3 summarizes the wavelengths and frequencies assigned to C–Se stretchings.

The progressive shift to longer wavelengths when going from methyldiselenide over diethyldiselenide and di-*iso*-propyldiselenide to di-*tert*-butyldiselenide should be pointed out. A similar shift has been observed in mercaptans, sulphides and disulphides (31), and Sheppard (32) interprets this in terms of changes in the force constants of the C–S linkages. This is in agreement with the theory of hyperconjugation put forward by the present author as a result of UV studies. The stronger the hyperconjugation, the larger force constant for the C–S or C–Se bond. Therefore dimethyldiselenide is to be expected to absorb at the highest frequency and di-*tert*-butyldiselenide at the lowest. An indication of the possible correctness of this interpretation is that diethyldiselenide and di-*n*-butyldiselenide have almost identical spectra in the UV (absorption maximum at 3115 and 3120 Å) as well as in the IR (as far as the C–Se bond is considered) with peaks at 18.67 and 18.60 μ respectively. Schotte's statement (33): "The question therefore arises if the anomalous ultraviolet absorption of di-*tert*-butyldisulphide could not in some way be correlated to the results of Sheppard that the C–S stretching frequency for *tert*-alkyl sulphides is appreciably lower than that of similar compounds with primary alkyl groups", is therefore appropriate.

This hyperconjugative effect is the only effect which with certainty can be detected from the present material of IR spectra of selenium compounds. The spectra of the carboxylic substituted diselenides as well as that of 6-selenoctic acid are too complicated for a certain frequency assignment to be possible. However the author regards it justifiable to present the IR spectra of some of the substances in view of the fact that there are so few experimental data from the long wavelength region. For comparison with diselenium-diacetic acid the spectrum of adipic acid is given in Fig. 13. Frequencies possibly associated with C–Se stretchings are given in Table 3.

Fig. 14. Infrared spectrum of 6-selenoctic acid. 15–40 μ . CsBr prism.(c) *Se–Se stretchings*

Since Se–Se stretchings are to be expected at about 40 μ it is not certain that they are detectable with the CsBr prism. As can be seen from the figures some bands are observed at the longest wavelengths, but no certain assignments can be done. Since, as Schotte (4) showed, the S–S band is displaced to shorter wavelengths when the S–S group is in a strained ring, the most favourable substance for detecting the Se–Se stretching should be 6-selenoctic acid. This substance actually does show some relatively strong bands at 35–38 μ which may be associated with Se–Se stretching.

Table 3. Infrared absorption bands probably due to C–Se stretchings.

Compound	λ (μ)	ν (cm^{-1})
Dimethyldiselenide	17.55	570
Diethyldiselenide	18.67	536
Di- <i>n</i> -butyldiselenide	18.6	537
Di- <i>iso</i> -propyldiselenide	19.35	517
Di- <i>tert</i> -butyldiselenide	19.70	507
Diselenium-diacetic acid	19.85	504
Diselenium- α -dipropionic acid	18.22	544
Diselenium- β -dipropionic acid	19.05	525
Diselenium- δ -di- <i>n</i> -valeric acid	19.8	505
1,2-diselenane-3,6-dicarboxylic acid	18.8	532
6-selenoctic acid	18.7	535

SUMMARY

The ultraviolet (2200–5500 Å) and infrared (15–40 μ) absorption spectra of some linear and cyclic diselenides have been recorded. Special attention has been paid to the interpretation of the spectral shifts caused by changes in the dihedral angle of the C–Se–Se–C group ("the steric effect"), the inductive effect of a carboxylic group and the hyperconjugative effect due to the

presence of hydrogen atoms in the neighbourhood of the Se-Se group ("the electronic effects"). Comparisons are made with the spectra of the corresponding sulphur compounds, which may be interpreted in an analogous way. The diselenides are more suited than the disulphides for a study in the ultraviolet region but in the infrared the reverse is true.

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Tryckt den 6 augusti 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

On the system $\text{SrO}_2\text{-H}_2\text{O-H}_2\text{O}_2$

1. The crystal structure of α - and β - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$

By NILS-GÖSTA VANNERBERG

With 5 figures in the text

Introduction

Earlier work

In connexion with investigations concerning the formation, structure, and reactions of inorganic peroxides and superoxides, the system $\text{SrO}_2\text{-H}_2\text{O-H}_2\text{O}_2$ has been investigated. Among the compounds which have been proved to exist in this system, the dihydroperoxidates with the formula $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$ have been studied with single crystal methods. Previously both these compounds as well as the corresponding barium and calcium compounds have been investigated with chemical methods by Riesenfeld and Nottebohm (1, 2). They have obtained the barium compound from a water solution of barium chloride and hydrogen peroxide by adding to it diluted ammonia. The calcium and strontium compounds were obtained when the corresponding peroxide octahydrates were treated with 30 % hydrogen peroxide solution at low temperature. The barium and calcium compounds were fairly pure, but the strontium compound could only be prepared as a 50 % mixture with the peroxide octahydrate. According to Riesenfeld and Nottebohm (1, 2) this was due to the extreme instability of the strontium compound.

General methods of preparation

After some trial syntheses the following procedure was found to be suitable for preparation of strontium and also of barium peroxide dihydroperoxidates. A certain amount of the metal nitrate was dissolved in a hydrogen peroxide solution. This solution was cooled to 0°C and a dilute ammonia solution was cautiously added. In this manner a solution supersaturated with the respective peroxide compound was obtained. Crystallization occurred very slowly. After about one hour the first crystals were visible. When they had reached a suitable size, they were filtered off and were washed with alcohol and ether. In this way rather good crystals suitable for X-ray single crystal investigations could be obtained. As will be mentioned later their properties were strongly dependent of the concentration of the metal ions, hydrogen peroxide, and ammonia.

X-ray methods

For the sake of phase identification powder photographs of Guinier type were taken of all preparations. The structural investigation was based on single crystal methods. The single crystals were rotated about suitable crystal axes and Weissenberg photographs were taken of the zero and first layer lines. Multiple film technique was used. The reflection intensities were estimated by visual comparison with known scales, and corrected with Lorentz and polarization factors. Since all crystals used in this investigation were very small no allowance was made for the absorption factor. The unit cell dimensions were obtained from Guinier photographs. $\text{CuK}\alpha$ radiation was used in all cases.

The system $\text{SrO}_2\text{-H}_2\text{O-H}_2\text{O}_2$

Established phases

In the system $\text{SrO}_2\text{-H}_2\text{O-H}_2\text{O}_2$ the existence of the following five compounds has been established.

Strontium peroxide, SrO_2

Strontium peroxide octahydrate, $\text{SrO}_2 \cdot 8\text{H}_2\text{O}$

Strontium peroxide monohydroperoxidate, $\text{SrO}_2 \cdot \text{H}_2\text{O}_2$

α -Strontium peroxide dihydroperoxidate, $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$

β -Strontium peroxide dihydroperoxidate, $\beta\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

Strontium peroxide has been prepared by dehydration of the peroxide octahydrate (1, 2) or from direct reaction between strontium oxide and oxygen (3). The peroxide octahydrate is precipitated when a diluted ammonia solution is added to a strontium nitrate solution in 3 % hydrogen peroxide. The other three compounds have not earlier been prepared in pure form. The crystal structure of strontium peroxide have been investigated by Bernal and co-workers (4). A preliminary examination of the octahydrate structure has been performed by King (5, 6).

Preparation and analysis of the compound $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$

0.5 g $\text{Sr}(\text{NO}_3)_2$ and 0.5 ml 25 % ammonia was dissolved in two flasks containing 20 ml 30 % H_2O_2 each. The solutions were cooled to 0°C and mixed cautiously. The supersaturated solution obtained was allowed to stand at 0°C for 20 hours. The crystals formed were filtered off and dried with alcohol and ether. The peroxide content was determined by titration with permanganate. The metal content was determined as oxide after ignition to 1000°C .

Analysis: Found: Sr^{2+} 46.2 %, O_2^{2-} 51.2 %. Calc.: Sr^{2+} 46.7 %, O_2^{2-} 50.6 %.

Preparation and analysis of the compound $\beta\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$

$\beta\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$ was obtained in a similar way. This time 1 g $\text{Sr}(\text{NO}_3)_2$ and 2 ml 25 % ammonia were dissolved in two portions of 20 ml 30 % hydrogen peroxide.

Analysis: Found: Sr^{2+} 46.2 %, O_2^{2-} 51.2 %. Calc.: Sr^{2+} 46.7 %, O_2^{2-} 50.6 %.

As the compounds could not be sufficiently dried, the Sr^{2+} analyses gave somewhat low values.

The structure of the α -strontium peroxide dihydroperoxidate

The single crystals of α - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$ consist of rather thick plates, where the crystallographic a and b axes coincide with the plate diagonals, while the c axis forms an angle of about 90° with the plate surface. The crystals are well developed, but twin formation is common. The α -compound is not stable at room temperature. The decomposition was usually total after about four hours. As the crystals could easily be adjusted, however, the time available was sufficient for the exposures.

The compound is monoclinic with the following dimensions of the unit cell

$$\begin{aligned} a &= 8.262 \pm 0.006 \text{ \AA} & c &= 8.050 \pm 0.005 \text{ \AA} \\ b &= 6.024 \pm 0.004 \text{ \AA} & \beta &= 79^\circ 28' \pm 8'. \end{aligned}$$

Calculated and observed $\sin^2\theta$ are tabulated in Table 1.

In all photographs only reflections from planes of the following types were observed:

$$\begin{aligned} hkl \text{ with } h+k &= 2n \\ h0l \text{ with } h &= 2n, l = 2n. \end{aligned}$$

Accordingly, possible space groups are $C 2/c$ no. 15 and Cc no. 9.

Table 1. X-ray powder diffraction data for α - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

$h k l$	$10^4 \cdot \sin^2\theta_{\text{obs}}$	$10^4 \cdot \sin^2\theta_{\text{calc}}$	$h k l$	$10^4 \cdot \sin^2\theta_{\text{obs}}$	$10^4 \cdot \sin^2\theta_{\text{calc}}$
1 1 0	253	254	3 3 0	2285	2284
1 1 1	314	315	4 2 1	2321	2326
2 0 0	360	360	1 1 5 }	2450	2457
0 0 2 }	381	380	5 1 2 }	2457	2457
1 1 1 }		382	5 1 1	2673	2679
1 1 2	700	701	0 4 1	2721	2715
0 2 1	748	748	4 2 2	2741	2746
2 0 2	874	875	1 1 5 }	2799	2795
3 1 1	968	968	2 2 4 }		2804
1 1 3	1005	1006	4 2 4	3076	3073
0 2 2	1034	1034	5 1 2	3137	3133
2 2 1	1042	1042	1 3 4	3211	3216
3 1 2	1152	1151	6 0 0	3237	3242
3 1 1	1171	1171	4 2 3	3359	3359
2 2 1	1179	1178	2 0 6	3376	3383
1 1 3	1208	1208	1 1 6	3465	3467
2 2 2	1259	1260	2 4 3	3631	3631
4 0 0	1442	1441	5 3 2	3763	3767
0 0 4	1519	1518	3 1 5	3846	3853
4 0 2	1549	1550	4 4 1 }	4020	4021
3 1 2 }	1560	1557	6 0 2 }		4027
1 3 0 }		1564	4 2 4	4163	4155
2 2 3	1667	1666	2 0 6 }	4180	4181
1 3 2	1876	1876	1 5 0 }		4184
1 1 4	1910	1907	4 4 1	4289	4291
1 3 2	2008	2011	7 1 1	4434	4435
4 2 1	2058	2056	1 1 7	4665	4665
3 1 3	2131	2132	3 5 1	4899	4898

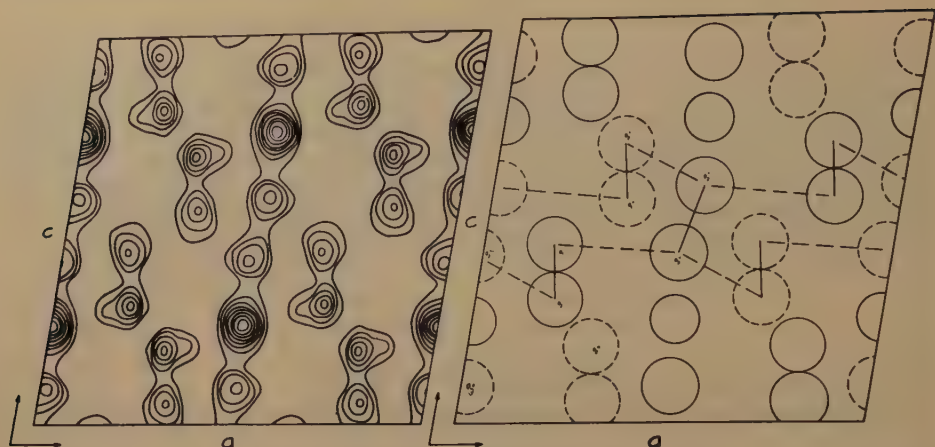


Fig. 1. (a) Electron density projection of $\alpha\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$ on the plane [010]. Electron density in arbitrary units. Every third equidensity line is drawn for the strontium atoms. (b) Schematic representation of the structure. Large full-drawn circles represent oxygen atoms with $y \approx 0$, dotted circles oxygen atoms with $y \approx \frac{1}{2}$.

As a consequence of the great instability of this compound, no determination of the specific gravity could be performed. However, if there were four formula units in the elementary cell, 70 % of the available space were filled up by ions. As the corresponding percentage for SrO_2 is 69 % and for $\text{H}_2\text{O}_2(s)$ 61 % the above assumption is probably correct. Moreover, the space groups calls for $4 \cdot n$ formula units. The only reasonable value of n is 1.

The Patterson projection of $\frac{1}{2}$ of the unit cell content on the [001] plane gave a half Sr-Sr vector and 3 Sr-O vectors. No other vectors were observed. This is only consistent with the space group $C 2/c$. This space group has five fourfold position sets. According to the Patterson projection the four strontium ions must be placed in (e) $0z\frac{1}{2}$; $0\bar{z}\frac{1}{2}$, where the parameter z was estimated to be 0.179. The evaluation of the positions of the strontium ions made it possible to determine most of the signs of the structure amplitudes. Using structure factors of the reflections $hk0$ and $h0l$ and $0kl$, three Fourier projections were made. From these (Fig. 1) was established that the 24 oxygens were located in three general (eightfold) positions and their approximate parameters could be evaluated.

The structure was refined until $R(h0l) = 0.18$, $R(hk0) = 0.21$, $R(0kl) = 0.21$, where $R = \sum |F_{\text{calc}}| - |F_{\text{obs}}| / \sum |F_{\text{obs}}|$. Observed structure factors have been corrected for thermal vibration by the factor $\exp[-B(\sin \theta / \lambda)^2]$ with $B = 2.3 \times 10^{-16} \text{ cm}^2$. The result arrived at is the following.

Atomic coordinates of $\alpha\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$.

	x	y	z
4Sr in (e)	0	0.179	$\frac{1}{2}$
8O ₁ in (f)	0.173	0.010	0.450
8O ₂ in (f)	0.196	-0.150	0.308
8O ₃ in (f)	0.015	0.520	0.088

Table 2. Observed and calculated structure factors for the hkl , $h0l$, and $0kl$ planes of α - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

hkl	F_{obs}	F_{calc}	hkl	F_{obs}	F_{calc}
200	100	+113	1004	39	+42
400	109	+137	006	86	-101
600	127	+147	206	78	-83
800	50	+57	406	121	-133
1000	77	+84	606	52	-74
110	35	+39	806	61	-61
310	39	-56	008	27	+35
510	41	+52	208	81	+96
710	20	+14	408	40	+76
910	14	+5	608	31	+48
020	11	+3	808	51	+95
220	63	-44	0010	36	-53
420	44	-46	2010	30	-60
620	28	-14	202	119	-124
820	46	-41	402	45	-51
1020	46	-37	602	82	-70
130	132	-133	802	86	-54
330	159	-125	204	88	+69
530	120	-110	404	85	+77
730	65	-69	604	123	+92
930	75	-83	804	90	+47
040	6	+10	206	146	-135
240	9	+8	406	57	-56
440	13	-10	606	115	-101
640	12	+7	208	60	+81
840	14	-3	408	65	+57
150	81	+54	020	17	+3
350	56	+32	021	71	-60
550	92	+54	022	165	+135
750	69	+46	023	42	+53
060	97	+112	024	57	-71
260	37	+59	025	97	-82
460	59	+71	026	35	+18
660	85	+81	027	86	+77
170	16	-2	028	49	-59
370	23	-29	029	55	-62
200	118	+113	040	6	+10
400	136	+138	041	72	+65
600	164	+147	042	60	+67
800	68	+57	043	121	-94
1000	117	+84	044	16	-24
002	95	-84	045	94	+71
202	39	-59	046	—	-14
402	144	-128	047	88	-74
602	71	-72	048	—	-16
802	83	-75	060	120	+112
1002	140	-94	061	76	-51
004	115	+100	062	87	-57
204	56	+74	063	39	+20
404	54	+69	064	86	+61
604	54	+53	065	64	-37
804	50	+67	066	68	-65

Table 3. Standard deviations in the atomic positions of $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

	x (Å)	y (Å)	z (Å)
Sr	0	0.01	0
O ₁	0.06	0.03	0.06
O ₂	0.07	0.10	0.10
O ₃	0.08	0.03	0.09

Table 4. Bond lengths and angles in $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

Sr'—Sr''	4.57 ± 0.01 Å
Sr'—Sr'''	5.11 ± 0.00
Sr—O ₂	2.65 ± 0.14
Sr—O ₃	2.44 ± 0.08
O ₁ '—O ₂ '	1.50 ± 0.13
O ₃ '—O ₃ '	1.48 ± 0.18
O ₁ '—O ₃ '	2.54 ± 0.10
O ₂ '—O ₃ '	2.67 ± 0.19
O ₂ '—O ₂ ''	3.23 ± 0.15
O ₁ '—O ₃ ''	3.21 ± 0.13
∧ O ₁ '—O ₃ '—O ₃ ''	103° ± 7°
∧ O ₃ '—O ₁ '—O ₂ '	88° ± 8°
∧ O ₃ '—O ₂ '—O ₁ '	107° ± 9°
∧ O ₂ '—O ₃ '—O ₃ ''	107° ± 9°
∧ the plane O ₁ 'O ₃ 'O ₃ ''	
—the plane O ₃ 'O ₃ 'O ₂ '	44°
∧ the plane O ₃ 'O ₂ 'O ₁ '	
—the plane O ₂ 'O ₁ 'O ₃ '	123°
∧ O ₂ '—the plane O ₁ 'O ₃ 'O ₃ ''	40°
∧ O ₁ '—the plane O ₂ 'O ₃ 'O ₃ ''	39°

The deviation of the last four angles is about 10°.

The comparison between the observed and calculated F -values is given in Table 2.

There is considerable overlap between oxygen atoms in the projections along the a and c axes, while the resolution is very good in the projection along the b axis. The standard deviations were calculated with Cruickshank's method (7) (Table 3). Distances and angles between the various atoms are tabulated in Table 4.

According to the crystallographic data obtained the $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$ is built up of Sr^{2+} ions in a double chain consisting of peroxide ions held together by strong hydrogen bonds. The distances between 2 oxygen atoms in the same peroxide group are 1.48 Å and 1.50 Å respectively. This is in good agreement with published data (8). The distances between oxygen atoms, held together by hydrogen bonds are 2.67 Å and 2.54 Å respectively. It is not impossible that this difference in the bond distance is real. The former value refers back to a part of the chain, where the peroxide ions are in

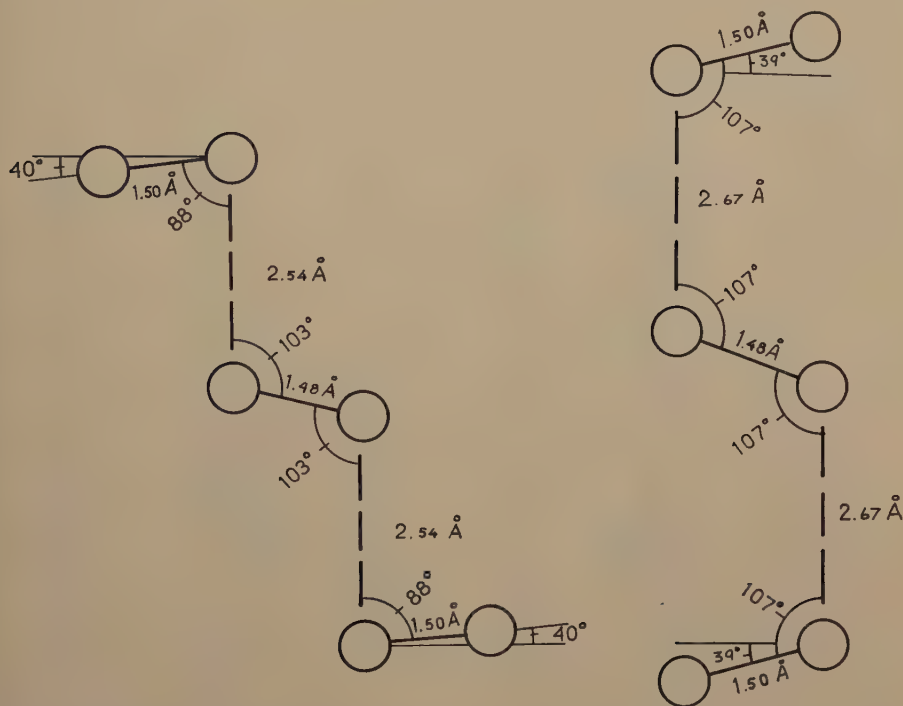


Fig. 2. A section of the peroxide double chain in $\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$. (a) The "trans" chain. (b) The "cis" chain.

a *cis*-position to the hydrogen bond, the later to a part, where the peroxide groups are in a *trans*-position to the hydrogen bond. See Fig. 2. The angle between the peroxide axis and the hydrogen bond direction is somewhat larger than the same angle in hydrogen peroxide, but this may be a consequence of the strong coulomb forces occurring in this structure. All the hydrogen atoms are involved in bond formation.

The angles between the two planes, containing the hydrogen bonds attached to a peroxide group, are 44° and 123°. The great similarity in the building units in the solid hydrogen peroxide lattice and in this structure can be seen in Fig. 3. The strontium ions are coordinated by four oxygen atoms arranged in a slightly deformed tetrahedron. The distances between oxygen and strontium are 2.44 Å and 2.65 Å respectively.

The structure of β -strontium dihydroperoxidate

Single crystals of β -strontium peroxide diperhydrate were obtained as previously described. The crystal needles are shaped in the form of lancets. None of the crystallographic axes coincide with the needle axis, so that the crystals had to be adjusted by the aid of Laue and oscillating photographs. As for the α -compound the crystals are not stable at room temperature, but the decomposition started not until after

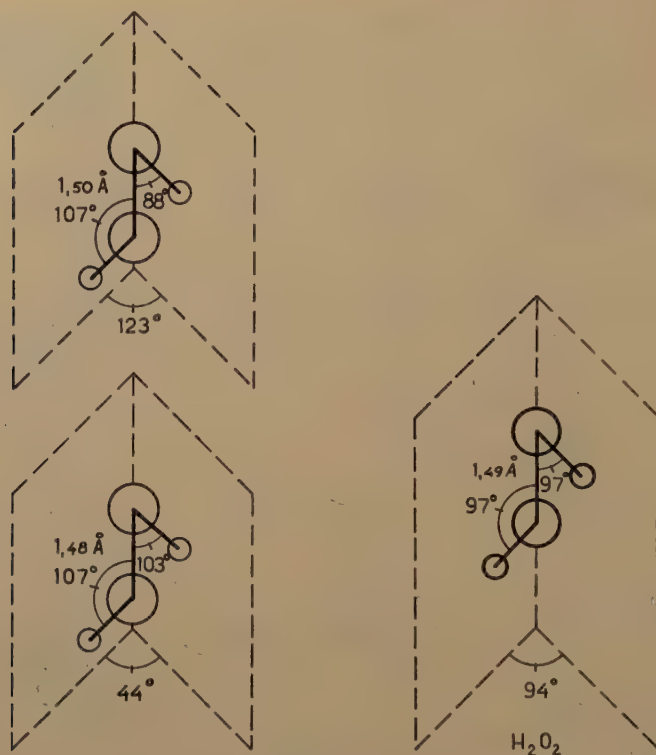


Fig. 3. (a) The peroxide units in $\alpha\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$; (b) the peroxide unit in H_2O_2 . The hydrogen atom positions in these figures are assumed to lie on the hydrogen bond directions.

seven hours. However, once started it was very rapid. The time available was mostly sufficient for the mounting, adjusting, and the exposure.

The compound was found to be monoclinic with following dimensions of the unit cell:

$$\begin{array}{ll} a = 7.715 \pm 0.004 \text{ \AA} & c = 6.015 \pm 0.003 \text{ \AA} \\ b = 8.754 \pm 0.005 \text{ \AA} & \beta = 86^\circ 20' \pm 2' \end{array}$$

The observed and calculated $\sin^2\theta$ are tabulated in Table 5.

The pattern obeys the same extinction rules as the pattern of the α -compound; accordingly, the space group must be $C 2/c$, or Cc . Also in this case the Patterson projections gave a verdict for the former space group, which requires four formula units in the elementary cell.

A Patterson projection on $[001]$ showed that the four strontium atoms must lie in $(e)0z\frac{1}{4}; 0\bar{z}\frac{3}{4}$. Probably by coincidence the parameter value was 0.179 also in this structure. When the positions of the strontium ions had been determined the signs of all the structure amplitudes could be obtained. Using the $hk0$ and hkl structure factors two Fourier projections were made (Fig. 4). From these projections it could be seen that the 24 oxygen atoms were located in three general (eightfold) positions.

Table 5. X-ray powder diffraction data for β - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$.

$h k l$	$10^4 \cdot \sin^2 \theta_{\text{obs}}$	$10^4 \cdot \sin^2 \theta_{\text{calc}}$	$h k l$	$10^4 \cdot \sin^2 \theta_{\text{obs}}$	$10^4 \cdot \sin^2 \theta_{\text{calc}}$
1 1 0	178	178	0 2 3	1793	1794
0 2 0	310	310	$\bar{2}$ 4 1	1840	1839
1 1 1	327	326	4 2 0	1914	1914
$\bar{1}$ 1 1	360	359	4 2 1	2014	2014
0 2 1	474	475	2 2 3	2098	2097
0 0 2	657	659	3 3 2	2164	2162
2 2 0	710	711	$\bar{3}$ 3 2	2361	2359
1 3 0	799	798	1 5 2	2667	2665
2 2 1	842	843	$\bar{5}$ 1 1	2833	2831
$\bar{1}$ 1 2	870	870	0 2 4	2946	2947
$\bar{2}$ 2 1	909	909	2 4 3	3031	3027
1 3 1	945	947	4 4 1	3076	3076
0 2 2	969	969	$\bar{2}$ 0 4	3168	3170
3 1 0	982	980	4 2 3	3202	3201
2 0 2	997	994	$\bar{2}$ 4 3	3226	3224
3 1 1	1094	1095	4 4 2	3371	3373
$\bar{2}$ 0 2	1123	1125	3 5 2	3401	3402
$\bar{3}$ 1 1	1191	1193	$\bar{5}$ 3 1	3453	3451
2 2 2	1305	1304	3 5 2	3601	3599
0 4 1	1405	1405	4 0 4	3979	3979
1 3 2	1425	1424	$\bar{5}$ 1 3	4307	4314
4 0 0	1604	1604	0 2 5	4434	4431
1 1 3	1612	1612	$\bar{3}$ 3 4		4435
$\bar{1}$ 1 3	1713	1711	2 6 3	4584	4579
2 4 1	1774	1773	4 6 4	4924	4924

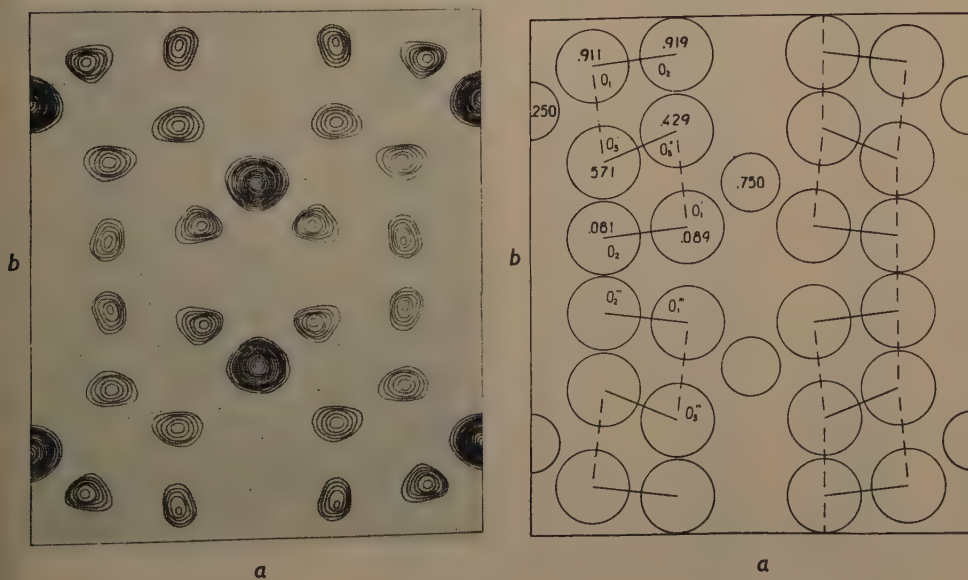


Fig. 4. (a) Electron density projection of β - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$ on the plane [001]. Electron density in arbitrary units. Every second equidensity line is drawn for the strontium atoms. (b) Schematic representation of the structure.

Table 6. Observed and calculated structure factors for $hk0$ and hhl planes of $\beta\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$.

hkl	F_{obs}	F_{calc}	hkl	F_{obs}	F_{calc}
200	50	+ 71	115	93	- 61
400	38	+ 41	116	35	- 33
600	149	+ 164	117	39	+ 55
800	70	+ 59	220	50	- 70
110	56	+ 64	221	91	- 121
310	56	+ 65	222	100	+ 97
510	—	+ 13	223	97	+ 68
710	35	+ 41	224	52	- 35
910	35	+ 48	225	55	- 57
020	83	- 82	226	38	+ 47
220	70	- 70	227	47	+ 59
420	86	- 71	330	83	- 110
620	57	- 52	331	16	+ 8
820	39	- 39	332	75	+ 87
130	133	- 118	333	—	+ 12
330	123	- 110	334	74	- 82
530	84	- 81	335	57	+ 45
730	75	- 73	336	69	+ 70
930	41	- 67	440	—	- 12
040	29	- 31	441	29	+ 40
240	24	- 31	442	20	+ 31
440	—	- 12	443	86	- 117
640	—	- 13	444	22	- 14
840	—	- 29	445	75	+ 71
150	64	+ 57	550	52	+ 72
350	115	+ 92	551	45	+ 52
550	78	+ 72	552	45	- 40
750	41	+ 39	553	49	- 69
060	26	+ 12	554	53	+ 37
260	135	+ 106	555	18	+ 17
460	114	+ 113	660	38	+ 32
660	38	+ 32	661	57	- 38
860	38	+ 58	662	71	- 68
170	—	+ 13	663	18	+ 20
370	35	- 26	664	92	+ 82
570	—	+ 25	770	—	+ 2
770	—	- 2	771	87	- 78
080	100	- 84	111	114	- 110
280	75	- 62	112	157	- 115
480	56	- 57	113	127	+ 108
680	58	- 67	114	—	- 0.2
190	70	- 58	115	83	- 69
390	47	- 52	116	31	- 33
590	49	- 58	117	68	+ 60
0100	51	+ 43	221	76	- 84
2100	21	+ 30	222	42	+ 49
1110	72	+ 67	223	41	+ 62
002	55	- 46	224	58	- 99
004	62	+ 56	225	74	- 74
006	133	- 129	226	34	+ 39
110	39	+ 64	227	70	+ 51
111	82	- 101	331	26	+ 26
112	—	+ 13	332	58	+ 92
113	140	+ 126	333	—	+ 8
114	56	+ 75	334	47	- 79

Table 6 (continued)

<i>h k l</i>	<i>F</i> _{obs}	<i>F</i> _{calc}	<i>h k l</i>	<i>F</i> _{obs}	<i>F</i> _{calc}
3 3 $\bar{5}$	43	+ 25	5 5 $\bar{2}$	94	- 72
3 3 $\bar{6}$	47	+ 69	5 5 $\bar{3}$	44	- 45
4 4 $\bar{1}$	94	+ 95	5 5 $\bar{4}$	53	+ 37
4 4 $\bar{2}$	—	+ 11	6 6 $\bar{1}$	44	- 30
4 4 $\bar{3}$	86	- 107	6 6 $\bar{2}$	95	- 65
4 4 $\bar{4}$	—	- 35	6 6 $\bar{3}$	—	- 7
4 4 $\bar{5}$	75	+ 43	6 6 $\bar{4}$	48	+ 58
5 5 $\bar{1}$	44	+ 45	7 7 $\bar{1}$	36	- 64

The structure was then refined by the aid of the “least square” method, until $R_{h k 0} = 0.14$ and $R_{h h l} = 0.19$. Calculated amplitudes have been corrected for thermal vibration $B = 2.9 \times 10^{-16}$ cm². The result is given below. See also Table 6.

Atomic coordinates for β -SrO₂·2H₂O₂

	<i>x</i>	<i>y</i>	<i>z</i>
4 Sr in (e)	0	0.179	$\frac{1}{4}$
8 O ₁ in (f)	0.143	0.093	0.911
8 O ₂ in (f)	0.332	0.073	0.919
8 O ₃ in (f)	0.331	0.220	0.429

Also here the standard deviations were calculated according to Cruickshank’s method (7). Table 7.

The structure of β -SrO₂·2H₂O₂ as determined here consists of Sr²⁺ ions and double chains of peroxide groups. Distances and angles between the various atoms are tabulated in Table 8. The peroxide groups are held together by strong hydrogen

Table 7. Standard deviations in the atomic positions of β -SrO₂·2H₂O₂.

	<i>x</i> (Å)	<i>y</i> (Å)	<i>z</i> (Å)
Sr	0	0.008	0
O ₁	0.04	0.04	0.09
O ₂	0.03	0.05	0.11
O ₃	0.07	0.03	0.08

Table 8. Bond lengths and angles in β -SrO₂·2H₂O₂.

Sr'—Sr''	4.34±0.01 Å	∧ O ₃ '—O ₁ '—O ₂ '	89°±5°
Sr'—Sr'''	4.90±0.01	∧ O ₁ '—O ₃ '—O ₃ ''	111°±6°
Sr—O ₁	2.38±0.11	∧ O ₂ '—O ₃ '—O ₃ ''	110°±4°
Sr—O ₃	2.56±0.10	∧ O ₃ '—O ₂ '—O ₃ ''	96°±4°
O ₁ '—O ₂ '	1.47±0.06	∧ the plane O ₂ ''' O ₃ ' O ₃ ''—the plane O ₃ ' O ₃ '' O ₁ '	47°
O ₃ '—O ₃ ''	1.55±0.15	∧ the plane O ₃ ' O ₂ ''' O ₁ '—the plane O ₂ ''' O ₁ ' O ₃ ''	128°
O ₁ '—O ₃ ''	2.64±0.12	∧ O ₁ '—the plane O ₂ ''' O ₃ ' O ₃ ''	34°
O ₂ '''—O ₃ '	2.56±0.08	∧ O ₂ '—the plane O ₁ O ₃ ' O ₃ ''	40°±6°
O ₂ '—O ₂ '''	3.26±0.17	the deviation of the last three angels is about 8°	
O ₂ '—O ₃ '	3.22±0.15		



Fig. 5. Part of the chain in $\beta\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$.

bonds. The distances between 2 oxygen atoms in a peroxide group are 1.47 Å and 1.55 Å. These differences are not significant. The corresponding distance for hydrogen peroxide is 1.49 Å (8). The distance between two oxygen atoms involved in the first hydrogen bond is 2.64 ± 0.10 Å. See Figs. 4 and 5. A hydrogen atom must be placed somewhere in the region between these oxygen atoms. This accounts for two of the four hydrogen atoms in $\beta\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$. The other two must be bonded to the oxygen atoms in $x=0.332$, $y=0.073$, $z=0.919$ and can form another hydrogen bond. The distance between this oxygen atom and the bonded atom in the next group is 2.56 Å. The angle between the peroxide axis and the hydrogen bond direction are 89° , 96° , 110° and 111° . The corresponding angle for hydrogen peroxide is 97° . The discrepancy, if real, is certainly due to the interaction of the strong electrostatic forces in this structure.

The angle between the two planes which contain the hydrogen bonds attached to a peroxide group, are 47° and 128° , in good agreement with the value for the α -compound.

The strontium ion is tetrahedrally coordinated to four oxygen atoms. However, the tetrahedron is not regular. The distances between oxygen and strontium are 2.38 Å and 2.56 Å respectively.

From Figs. 2 and 5 it is clear that the "cis chain" in $\alpha\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$ is very similar to one of the chains in $\beta\text{-SrO}_2\cdot 2\text{H}_2\text{O}_2$. Furthermore it is interesting to note that

these two groups are very similarly placed in the lattice. This is readily seen if the axes are interchanged in the following way: The a axes in the α -compound correspond to the b axes in the β -compound, the b axes in α to the c axes in β , and the c axes in α to the a axes in β .

SUMMARY

α - and β - $\text{SrO}_2 \cdot 2\text{H}_2\text{O}_2$ have been prepared and their crystal structures have been investigated. The following crystallographic data were evaluated

α		$\alpha\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$		$\beta\text{-SrO}_2 \cdot 2\text{H}_2\text{O}_2$		
Space group		C 2/c No. 15		C 2/c No. 15		
a		$8.262 \pm 0.006 \text{ \AA}$		$7.715 \pm 0.004 \text{ \AA}$		
b		$6.024 \pm 0.004 \text{ \AA}$		$8.754 \pm 0.005 \text{ \AA}$		
c		$8.050 \pm 0.005 \text{ \AA}$		$6.015 \pm 0.003 \text{ \AA}$		
β		$79^\circ 28' \pm 8'$		$86^\circ 20' \pm 2'$		
4Sr (e)	0	0.179	$\frac{1}{4}$	0	0.179	$\frac{1}{4}$
8O (f)	0.173	0.010	0.450	0.143	0.093	0.911
8O (f)	0.196	-0.150	0.308	0.332	0.073	0.919
8O (f)	0.015	0.520	0.088	0.331	0.220	0.429

The α - and β -compound contains infinite double chains of peroxide groups extended in the [110] and [011] plane.

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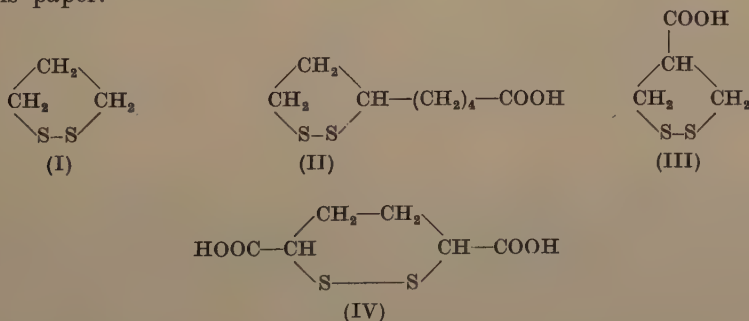
The strain in the 1,2-dithiolane ring

By GÖRAN BERGSON and LENNART SCHOTTE¹

With 4 figures in the text

Introduction

The 1,2-dithiolane ring (I) became an important unit in modern sulphur chemistry when its presence in 6-thioctic acid (α -lipoic acid) (II) was discovered (1). The hypothesis advanced by Calvin and co-workers (2) about the rôle of 6-thioctic acid in the photosynthesis has caused many efforts to determine the strain in the 1,2-dithiolane ring. A necessary condition for Calvin's hypothesis to be true is that this strain is at least about 20–25 kcal/mole. Calvin estimates the strain as 27 kcal/mole from UV data, as 6 kcal/mole from equilibrium measurements and as at least 11 kcal/mole from conformation analysis (3). Sunner (4) has reported a value of 4–5 kcal/mole from thermochemical measurements. The present authors have performed a conformation analysis of the 1,2-dithiolane ring in 1,2-dithiolane-4-carboxylic acid (III), prepared by Schotte and Ström (5), on the basis of a structure determination by Foss (6). As a minimum for the strain we obtain 16 kcal/mole. A critical discussion of the data is given at the end of this paper.



Analysis of the ring in 1,2-dithiolane-4-carboxylic acid

A. General formula for the strain

We suppose that the strain is due to three factors, viz. (1) stretching of valence bonds; (2) bending of valence angles; (3) torsion about the bonds.

The strain energy, E_{strain} , is thus a sum of three terms:

¹ Both authors contribute equally.

$$E_{\text{strain}} = E_{\text{stretching}} + E_{\text{bending}} + E_{\text{torsion}} \quad (\text{a})$$

The stretching and bending energies are supposed to follow the usual square laws, and the torsions a cosine law:

$$E_{\text{stretching}} = \frac{1}{2} k_r (\Delta x_r)^2; \quad E_{\text{bending}} = \frac{1}{2} k_\alpha (\Delta \omega_\alpha)^2; \quad (\text{b})$$

$$E_{\text{torsion}} = \frac{1}{2} V_r (1 - \cos n \Delta \theta_r). \quad (\text{c})$$

Thus:

$$E_{\text{strain}} = 7.20 \cdot 10^{-8} \sum_r k_r (\Delta x_r)^2 + 2.19 \cdot 10^9 \sum_\alpha k_\alpha (\Delta \omega_\alpha)^2 + \frac{1}{2} \sum_r V_r (1 - \cos n \Delta \theta_r), \quad (\text{d})$$

where

E_{strain} = the strain energy in *kcal/mole*.

k_r = the stretching force constant for the bond r in *dyn/cm*.

k_α = the bending force constant for the angle α in *erg/radian²*.

V_r = the torsion barrier about the bond r in *kcal/mole*.

Δx_r = the deviation from normal bond length in *cm* $\cdot 10^{10}$.

$\Delta \omega_\alpha$ = the deviation from normal bond angle in *degrees*.

$\Delta \theta_r$ = the deviation from normal dihedral angle.

n = 2 for the S-S bond and $n=3$ for the C-C bonds.

In this theory the molecule is regarded from the point of view of classical mechanics. With such a model, equations (b) are usually supposed to give a good estimate of the steric effect for small deviations (7). Some remarks regarding equation (c) may be of interest. It has been shown empirically that the cosine law better fits the experimental results for large deviations than the square law (8). With the help of elementary trigonometry, however, it is realized that the two laws approach each other for small deviations. It must also be pointed out that equation (c) has a good theoretical background, when S-S bonds are considered. Bergson (9) has deduced a quantum mechanical expression for the torsion barrier of the sulphur-sulphur bond:

$$\frac{4S}{1-S^2} (E_{\text{AS}} - \beta).$$

If S^2 ($=0.0166$) is neglected, and if the angular dependence of the integrals S and β is considered, it is easily verified that the quantum mechanical expression for rotation about the S-S bond is identical with the formula (c). Regarding the torsion about the C-C bonds the situation is less clear. The torsion barrier is only partly due to van der Waals interactions between the hydrogen atoms, and Kauzmann (10) states: "Its origin is obscure." It must also be pointed out that we have neglected torsion about the C-S bonds. As far as we have found, nothing is known about it.

B. Assignments of normal bond lengths, bond angles and dihedral angles

In order to be able to apply our formula for the strain we must first determine the quantities Δx_r , $\Delta \omega_\alpha$ and $\Delta \theta_r$. Therefore it is necessary to know the real and the normal values for the bond lengths, x_r , bond angles ω_α and

Table I. Structural parameters.

Bond or angle	x_r or ω_α (Normal value)	x_r or ω_α (Found in 1,2-dithiolane- 4-carboxylic acid)	θ (Normal value)	θ (Found)
C—C	1.54	1.55 ± 0.03 ; 1.48 ± 0.025 (Å)	60°	$20^\circ \pm 10^\circ$
C—S	1.81	1.83 ± 0.02 ; 1.85 ± 0.02 (Å)	—	—
S—S	2.08	2.096 ± 0.009 (Å)	90°	$26.6 \pm 1^\circ$
	109.5°	$114.2^\circ \pm 1.6^\circ$	—	—
	109.5°	$104.2^\circ \pm 1.3^\circ$; $110.6^\circ \pm 1.2^\circ$	—	—
	107.0° 90.0°	$92.6^\circ \pm 0.7^\circ$; $96.6^\circ \pm 0.6^\circ$	—	—

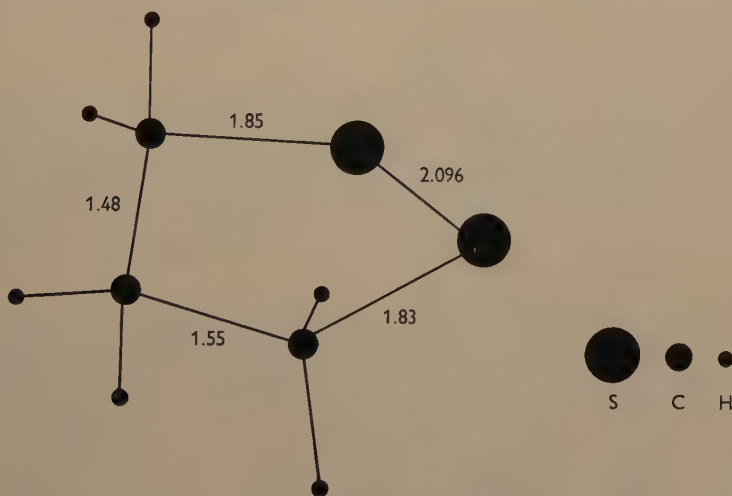


Fig. 1. Model of the ring in 1,2-dithiolane-4-carboxylic acid. Bond distances in Å units. The carboxylic group is replaced by a hydrogen atom.

dihedral angles θ_r . The real values for x_r and ω_α as determined by Foss are given in Table 1, column 3. The real values for the dihedral angles are given in column 5. The sulphur-sulphur dihedral angle is that given by Foss, and the angle for the carbon-carbon bonds have been estimated from a model based on Foss' investigation (Fig. 1) and the assumption that the H—C—H bond angle is 109.5° . The normal bond lengths have been defined as the sum of Pauling's covalent bond radii, and the normal carbon valence angle is assumed to be 109.5° . It is more difficult to assign a normal valence angle for sulphur. We have done this in two different ways. The first (called *alt. I* below) takes the bond angle in dimethyldisulphide (107°) as the normal value (11), and the other (*alt. II*) assumes pure *p*-bonding and the valence angle 90° . The normal dihedral angle for the sulphur bond is 90° , and for the C—C bonds 60° . The dihedral angle for sulphur bonds is defined as the angle between the planes CSS and SSC, and for carbon bonds as the smallest angle between the planes HCC and CCH. Figures 2 and 3 illustrate the meaning of the dihedral angles.

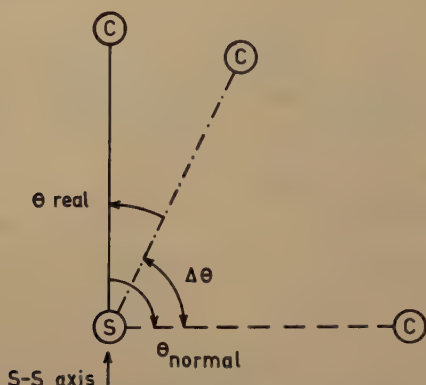


Fig. 2. Dihedral angles at the S-S bond.

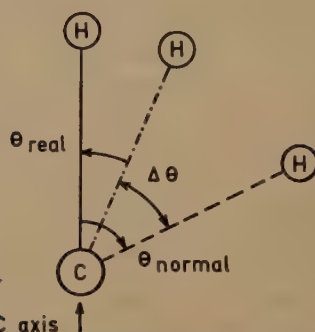


Fig. 3. Dihedral angles at the C-C bond.

C. The force constants and torsion barriers

In order to get a numerical result we must also know the force constants and torsion barriers in equation (d). The values we have used are summarized in Table 2, and only a few remarks seem necessary. The stretching force constants for the C-S and S-S bonds have been calculated from IR frequencies recorded by Schotte for some 5-membered cyclic disulphides (14), with the assumption of simple harmonic motion.

Regarding the torsion about the S-S bond the barrier found for non-cyclic compounds (12 ± 2 kcal/mole) has been used in *alt. I* (sulphur valence angle 107°). In *alt. II*, the barrier 14.16 ± 0.19 kcal/mole has been used. This value has been calculated from the UV absorption by means of Bergson's theory (which is based on pure *p*-orbitals) with the correct value of $26.6^\circ \pm 1^\circ$ for the dihedral angle.

D. Some remarks to the numerical calculations

The details of the numerical calculation are given in Table 3. We have performed the calculation both for maximum and minimum deviations, and the

Table 2. The force constants and the torsion barriers.

Bond or angle	k_r or k_α	V_τ	Method and references
C—C	$4.5 \cdot 10^5$	2.8	Raman, Herzberg (12) Thermodynamic, Pitzer (13)
C—S	$2.3 \cdot 10^5$	—	IR, $\nu = 664 \text{ cm}^{-1}$, Schotte (14) and this work
S—S	$2.7 \cdot 10^5$	12 ± 2 14.16 ± 0.19	IR, $\nu = 539 \text{ cm}^{-1}$, Schotte (14) and this work Raman, Scott <i>et al.</i> (15) Theor. calc. from UV, Bergson (9) and this work
	$0.8 \cdot 10^{-11}$	—	Raman, Kilpatrick, Pitzer and Spitzer (16)
	$0.92 \cdot 10^{-11}$	—	Raman, Scott <i>et al.</i> (15)
	$1.06 \cdot 10^{-11}$	—	Raman, Scott <i>et al.</i> (15)

Table 3. Details of the numerical calculation.

α	k_r or k_α	V_r	Δx_r or $\Delta \omega_\alpha$ max	Δx_r or $\Delta \omega_\alpha$ min	$\Delta \theta_r$	$E_{\text{stretching}}$	E_{bending}	E_{torsion}
C	$4.5 \cdot 10^5$	2.8	4; 8.5	0; 3.5	40 ± 10	1.63 ± 1.23	—	4.01 ± 1.21
S	$2.3 \cdot 10^5$	0	4; 6	0; 2	—	0.46 ± 0.40	—	—
S	$2.7 \cdot 10^5$	12 ± 2	2.5	0.7	63.4 ± 1	0.07 ± 0.06	—	9.62 ± 1.76
		14.16 ± 0.19						12.04 ± 0.68
C	$0.8 \cdot 10^{-11}$	—	6.3	3.1	—	—	0.44 ± 0.27	—
S	$0.92 \cdot 10^{-11}$	—	6.6; 2.3	4.0; 0.1	—	—	0.66 ± 0.33	—
S	$1.06 \cdot 10^{-11}$	—	15.1; 11.0 3.3; 7.2	13.7; 9.8 1.9; 6.0	—	—	7.33 ± 0.74 1.19 ± 0.27	—

energies given in columns 7-9 are the arithmetrical means together with the limits of error thus obtained. We have not investigated whether the geometrical requirements of the ring allow an addition of all the errors, and our calculations are therefore perhaps too pessimistic. The most important thing is, however, that our lowest value does not exceed the true value, and our method of calculation gives a guarantee for this. In Table 4 the calculations have been summarized.

E. The results of the analysis

The results of our conformation analysis are given in Table 4. With *alt. I* the strain energy is 24.2 ± 6 kcal/mole and with *alt. II* 20.5 ± 4.4 kcal/mole. It is therefore evident that in whichever manner the calculations are performed, the strain energy is at least 16 kcal/mole. It must also be pointed out that the largest contribution to the strain comes from the torsion about the single bonds. The stretching energy is as small as 2.2 ± 1.7 kcal/mole which is in agreement with results obtained from conformation analysis in general (7). The bending energy is rather large, 8.4 ± 1.3 kcal/mole if the normal sulphur valence angle is 107° , but if this angle is put equal to 90° , the bending energy is only 2.3 ± 0.9 . It seems difficult to us to choose between the two alternatives, but a glance at the net results shows that it is of no fundamental importance, in view of other inherent uncertainties in such a calculation.

Discussion

A primary question is whether the conformation analysis of the ring in 1,2-dithiolane-4-carboxylic acid can be applied to the 1,2-dithiolane ring in other

Table 4. Summary of the numerical calculations.

	$E_{\text{stretching}}$	E_{bending}	E_{torsion}	E_{strain}
Alt. I	2.16 ± 1.69	8.43 ± 1.33	13.63 ± 2.97	24.22 ± 5.99
Alt. II	2.16 ± 1.69	2.29 ± 0.85	16.05 ± 1.89	20.50 ± 4.43

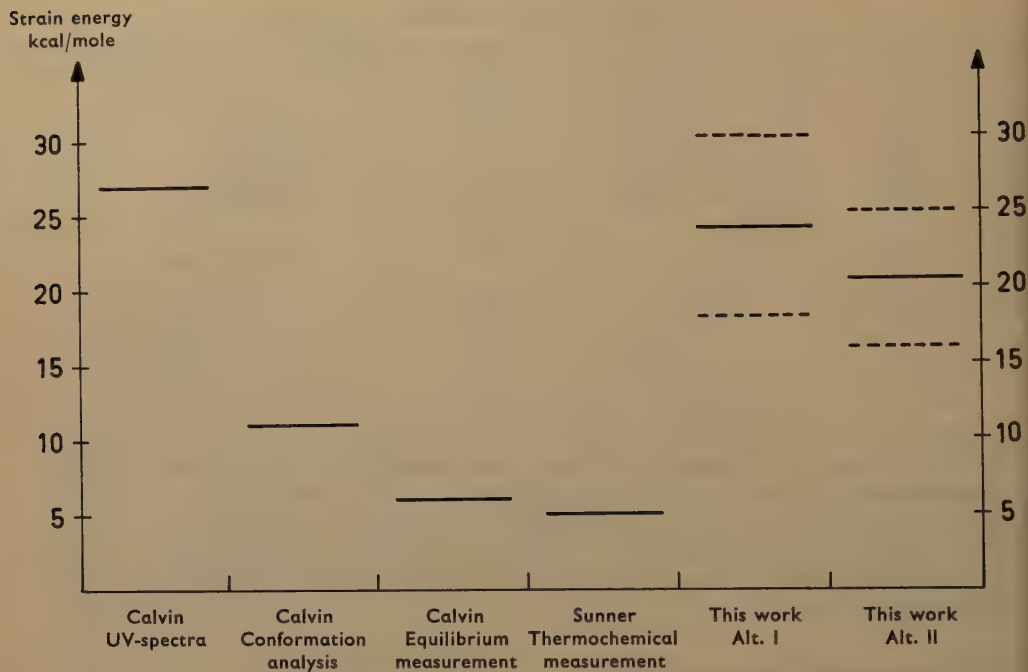


Fig. 4. Determinations of the strain energy in the 1,2-dithiolane ring.

substances, particularly in 6-thioctic acid. A definite answer cannot of course be given, since it would require the knowledge of the exact structural parameters in those substances. It is evident that one can imagine the 1,2-dithiolane ring to assume a whole set of different conformations and that one of these corresponds to a smallest strain energy. It is difficult, however, to assign to the ring in the 4-carboxylic acid, as well as in any dithiolane derivative, a conformation corresponding to a higher strain. As far as the method of conformation analysis is correct, we can therefore state that *the strain in 1,2-dithiolane derivatives is equal to or larger than 16 kcal/mole*. The exact value is determined by the geometrical requirements of the ring, but it must fall within the region 16–30 kcal/mole. We will therefore compare our results with the values obtained by other methods applied on 1,2-dithiolane itself and 6-thioctic acid mentioned in the introduction. Figure 4 gives a summary of all determinations of the strain energy.

The strain 27 kcal/mole is based on the observation that 1,2-dithiolane derivatives have their UV absorption maximum at 3300 Å, whereas normal disulphides absorb at 2500 Å. 27 kcal/mole is now the difference in excitation energy. Calvin is quite aware of the arbitrariness in such a procedure, and Bergson (9) has recently given a more detailed analysis of the meaning of the light absorption differences. Because of the Franck-Condon principle, stretching and bending energies together with the strain energies due to torsion about the C–C bonds cannot be observed in UV spectra, and the only thing which is recorded is the torsion about the S–S bond. This torsion energy, however, changes sign when

going from the ground to the excited state, and 27 kcal/mole is the sum of an energy loss in the ground state and an energy gain in the excited state. Estimations of steric strain from UV data therefore have no real significance.

Calvin's value 6 kcal/mole is based upon equilibrium measurements between 1,2-dithiolane and toluene- α -thiol, which is a straightforward method. Unfortunately, this investigation was only a preliminary one, and Calvin himself says that the value cannot be taken too seriously.

Sunner's value 4-5 kcal/mole is based upon calorimetric measurements of the oxidation of mercaptanes, and this method is also straightforward. The very low values obtained by Sunner and Calvin in their experimental determinations might be due to the formation of polymeric material. The extreme care which must be taken in experimental work with 1,2-dithiolane and its derivatives is evident from the investigation by Bergson and Claeson on the reaction polymer $\rightleftharpoons$ monomer (17).

Calvin's conformation analysis, which gave a strain of at least 11 kcal/mole, was not based upon real structural parameters but was only a rough estimation.

In conclusion it can be said that the question of steric strain in the 1,2-dithiolane ring cannot be regarded as settled. The results of the conformation analysis presented in this paper indicate that more experimental work is needed. An accurate direct determination of the strain in 1,2-dithiolane-4-carboxylic acid would further give valuable information about the validity of conformation analysis.

As far as steric strain is concerned it is, however, *our opinion that Calvin's hypothesis of the rôle of 6-thioctic acid in photosynthesis cannot be disregarded at present.* To give the final answer is a matter for experimental biochemistry.

It is moreover of fundamental importance to mention that the strain in the six-membered disulphide ring in 1,2-dithiane-3,6-dicarboxylic acid (IV) by means of the same numerical method has been determined as at least 5 kcal/mole. Even in this case the largest contribution to the strain, at least 3 kcal/mole, comes from the deviation in dihedral angle for the sulphur bond. The difference in strain energy for the five- and six-membered disulphide ring is significant and supports earlier observations about the special character of the 1,2-dithiolane derivatives.

SUMMARY

An estimate of the steric strain in the 1,2-dithiolane ring has been performed by means of classical conformation analysis. The calculations are based upon a structure determination of 1,2-dithiolane-4-carboxylic acid by Foss. The results indicate a strain of 16-30 kcal/mole. Previous estimates of the strain are discussed.

ACKNOWLEDGEMENTS

The present investigation was made possible thanks to the structure determination performed by Professor Olav Foss, University of Bergen. We are greatly indebted to him for his interest in our problems and for putting his results at our disposal prior to publication.

We also wish to thank Professor Arne Fredga for the facilities placed at our disposal.

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Department of Organic Chemistry, Chemical Institute, University of Uppsala. March 1958.

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Chelate alkali compounds in organic reactions. III

On the reactivity of ions and ion-pairs (chelates) in the methylation of ethyl acetoacetate

By ARNE BRÄNDSTRÖM

With 2 figures in the text

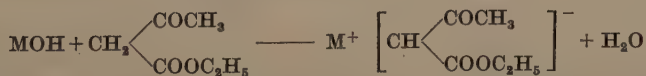
In the first paper (1) of this series a theory has been presented for the alkylation reaction of alkali compounds of various substances as being a reaction of both the ions and the ion-pairs (often chelated).

In the second paper (2) it was demonstrated that both the ion and the ion-pair (chelated) are reactive in the methylation of ethyl acetoacetate.

In the present paper this line of investigation is continued by kinetic measurements of the methylation of both the ions and the ion-pairs (chelated) of ethyl acetoacetate. This has been possible by determining the velocity constant at several different initial concentrations and applying the method of calculation recently published by the present author (3).

The velocity of the methylation was measured in dry ethanol for the alkali compounds of ethyl acetoacetate. The water content was about 0.02 per cent and the alcohol was protected from contaminations from carbon dioxide as far as possible.

In this solvent it was found that the saponification reaction due to the water content was completely eliminated for both the sodium and the lithium compound. Even if the water content of the solvent was as high as 1 per cent, a 0.1 *M* solution of the sodium compound of ethyl acetoacetate was only very slowly saponificated. This clearly demonstrates that for the sodium and the lithium compound the equilibrium is almost completely displaced to the right in an alcoholic solution, for the free ester ought to be rapidly saponificated in contrast to the alkali compound of the ester.



The potassium compound was, on the other hand, slowly saponificated to the potassium salt of acetoacetic acid. This reaction was most pronounced in the dilute solutions and prevented kinetic measurements in solutions weaker than about 0.02 *M*. The saponification reaction of the potassium compound of ethyl acetoacetate also explains why the measurements of the electrolytic conductivity of this compound by White (4) gives a limiting conductivity which, when compared with that obtained for the sodium compound, is inconsistent with the law of independent migration of the ions.

Table 1. The conductivity of sodium ethyl acetoacetate in ethanol at 25°C (4).

$$K = 1.31 \times 10^{-3}, \Lambda_0 = 43.1.$$

$\sqrt{c}$	c	Λ	α	$1/\alpha\Lambda_0 \times 10^2$	γ	$\alpha c \gamma^2 \times 10^4$	$1/\Lambda_0 \times 10^2$
0.5000	0.2500	3.103	0.0966	—	—	—	—
0.3536	0.1250	4.712	0.1431	—	—	—	—
0.2500	0.0625	6.691	0.1973	—	—	—	—
0.1768	0.03125	8.96	0.2559	—	—	—	—
0.1250	0.01563	12.288	0.3413	6.798	0.684	24.96	2.365
0.08839	0.007813	15.81	0.4265	5.440	0.729	17.71	2.295
0.06250	0.003906	19.71	0.5175	4.484	0.774	12.11	2.333
0.04419	0.001953	24.34	0.6242	3.717	0.813	8.06	2.286
0.03125	0.0009767	28.74	0.7216	3.215	0.852	5.12	2.306

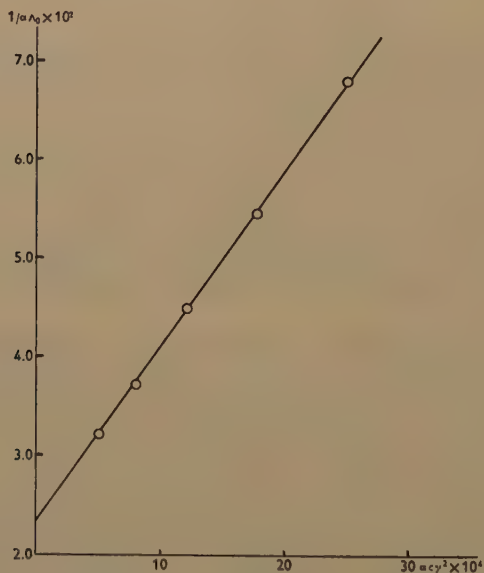


Fig. 1. The conductivity of sodium ethyl acetoacetate in ethanol at 25°C.

The measurements by White (4) (Table 1, Fig. 1) of the electrolytic conductivity of the sodium compound of ethyl acetoacetate in dry ethanol are not disturbed by the saponification reaction and when plotted by the method given by Brändström (3) they give a limiting conductivity $\Lambda_0 = 43.1$ and a dissociation constant of the ion-pair $K = 1.31 \times 10^{-3}$. This dissociation constant is much lower than that for the ion-pair of sodium ethoxide, $K = 9.54 \times 10^{-3}$. This means that the sodium ion and the ethyl acetoacetate anion are much closer together than the ions of the sodium ethoxide ion-pair. It is hard to find any other explanation for this than that the ion-pair of the sodium compound of ethyl acetoacetate is chelated.

A measurement of the electrolytic conductivity shows for the lithium compound

of ethyl acetoacetate (Table 2) that this substance has a very low conductivity which is only slowly changed with concentration. This indicates a strong chelation and a formation of higher associated complexes. A possible existence of such complexes which may be electrolytically conducting, e.g. triple ions, makes a calculation of the degree of dissociation highly uncertain.

The velocity constants K_n were measured for the reaction between the sodium compound of ethyl acetoacetate and methyl iodide in dry ethanol at 6 different initial concentrations of the sodium compound, varying from $M/4$ to $M/128$ (Tables 3–8).

Table 2. The conductivity of Lithium ethyl acetoacetate in ethanol at 25°C.

c	0.2500	0.1250	0.0625	0.03125	0.01563	0.007813
Λ	0.879	1.095	1.397	1.790	2.471	3.529

Tables 3–9. The reaction $(\text{CH}_3\text{COCHCOOC}_2\text{H}_5)\text{Na} + \text{CH}_3\text{I}$.

Table 3

$$K_n = 0.0863$$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.2509	0.4009	—
5.12	0.2116	0.3616	0.0291
10.08	0.1824	0.3324	0.0571
15.08	0.1597	0.3097	0.0841
20.00	0.1403	0.2903	0.1122
25.00	0.1229	0.2729	0.1429
30.25	0.1091	0.2591	0.1721
35.07	0.0990	0.2490	0.1970
40.00	0.0891	0.2391	0.2252
45.37	0.0796	0.2296	0.2565
50.80	0.0727	0.2227	0.2826

Table 4

$$K_n = 0.0875$$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.1255	0.4009	—
5.00	0.1068	0.3822	0.0492
10.08	0.0905	0.3659	0.1024
15.08	0.0772	0.3526	0.1550
19.97	0.0663	0.3417	0.2076
24.92	0.0564	0.3318	0.2651
29.82	0.0488	0.3242	0.3179
34.97	0.0425	0.3179	0.3694
40.00	0.0371	0.3125	0.4210
44.88	0.0334	0.3088	0.4615
49.95	0.0290	0.3044	0.5166

Table 5

$$K_n = 0.0893$$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.06331	0.4058	—
5.08	0.05386	0.3964	0.0599
9.95	0.04547	0.3880	0.1242
15.18	0.03750	0.3800	0.1989
19.98	0.03218	0.3747	0.2594
25.15	0.02726	0.3698	0.3255
29.98	0.02313	0.3656	0.3920
34.87	0.01987	0.3624	0.4541
39.93	0.01689	0.3594	0.5211
44.97	0.01399	0.3565	0.5993
50.12	0.01215	0.3547	0.6584

Table 6

$$K_n = 0.0911$$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.03166	0.4058	—
4.87	0.02754	0.4016	0.0561
10.07	0.02263	0.3967	0.1361
14.92	0.01907	0.3932	0.2066
19.83	0.01590	0.3900	0.2819
24.82	0.01327	0.3874	0.3576
30.00	0.01121	0.3853	0.4285
34.87	0.00941	0.3835	0.5024
39.90	0.00799	0.3821	0.5718
45.08	0.00643	0.3805	0.6644

Table 7
 $K_n = 0.0944$

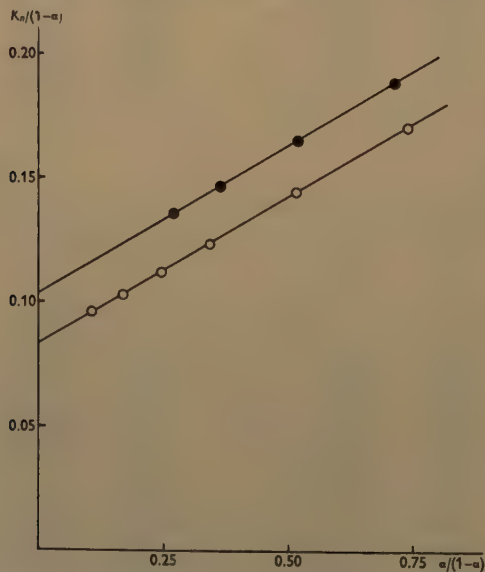
$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.01589	0.4047	—
4.97	0.01296	0.4018	0.0857
9.93	0.01071	0.3995	0.1658
14.93	0.00882	0.3976	0.2480
19.95	0.00733	0.3961	0.3268
25.10	0.00609	0.3949	0.4059
30.02	0.00506	0.3939	0.4852
35.00	0.00425	0.3931	0.5602
40.18	0.00341	0.3922	0.6548
44.85	0.00286	0.3917	0.7305
49.92	0.00244	0.3912	0.7990

Table 8
 $K_n = 0.0972$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.007945	0.4047	—
5.03	0.006518	0.4033	0.0846
10.30	0.005327	0.4021	0.1708
14.98	0.004434	0.4012	0.2494
20.02	0.003596	0.4004	0.3397
25.13	0.002968	0.3998	0.4223
29.92	0.002464	0.3993	0.5025
34.93	0.002086	0.3989	0.5745
40.02	0.001697	0.3985	0.6637
44.93	0.001380	0.3982	0.7532
49.93	0.001166	0.3979	0.8261

Table 9
 $K_n = 8.30 \times 10^{-2}$, $K_i = 11.67 \times 10^{-2}$.

c	α	K_n		$K_n/(1-\alpha)$	$\alpha/(1-\alpha)$
		found	calc.		
0.2500	0.0966	0.0863	0.0863	0.0955	0.1069
0.1250	0.1431	0.0875	0.0878	0.1021	0.1670
0.06250	0.1973	0.0893	0.0896	0.1112	0.2458
0.03125	0.2559	0.0911	0.0916	0.1224	0.3437
0.01563	0.3413	0.0944	0.0945	0.1433	0.5177
0.007813	0.4265	0.0972	0.0974	0.1695	0.7428

Fig. 2. The reaction $[\text{CH}_3\text{COCHCOOC}_2\text{H}_5]\text{M} + \text{CH}_3\text{I}$. ●, $K = \text{M}$; ○, $\text{Na} = \text{M}$.

In all cases the initial concentration of methyl iodide was about 0.40 *M*. The degree of dissociation α of the sodium compound was calculated from the conductivity values by White. A plot of $K_n/(1-\alpha)$ against $\alpha/(1-\alpha)$ gives a straight line from which the velocity constants for the free ethyl acetoacetate anion $K_i = 0.117$ and that for the chelated ion pair $K_m = 0.083$ can be calculated (Table 9, Fig. 2).

The velocity constants K_n were measured for the reaction between the lithium compound of ethyl acetoacetate and methyl iodide in dry ethanol at the same concentrations as those used for the sodium compounds (Tables 10–15). Since at present a calculation cannot be made of the degree of dissociation from the conductivity data the values of K_i and K_m cannot be calculated for the lithium compound. From the values obtained of K_n (Table 16) the value of K_m can be supposed to be about 0.012, i.e. considerably less than that for the sodium compound.

Tables 10–16. The reaction $(\text{CH}_3\text{COCHCOOC}_2\text{H}_5)_2\text{Li} + \text{CH}_3\text{I}$.

Table 10
 $K_n = 0.01332$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.2484	0.4068	—
9.82	0.2337	0.3921	0.0103
19.97	0.2225	0.3809	0.0193
30.12	0.2097	0.3681	0.0301
39.93	0.2014	0.3598	0.0377
49.92	0.1912	0.3496	0.0478
60.05	0.1835	0.3419	0.0560
70.15	0.1750	0.3334	0.0655
79.97	0.1670	0.3254	0.0755
89.90	0.1604	0.3188	0.0840
99.95	0.1544	0.3128	0.0924

Table 11
 $K_n = 0.01355$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.1242	0.4068	—
10.25	0.1164	0.3990	0.0200
19.97	0.1107	0.3933	0.0354
30.20	0.1047	0.3873	0.0527
40.03	0.0991	0.3817	0.0704
50.10	0.0943	0.3769	0.0864
60.02	0.0898	0.3724	0.1024
70.08	0.0854	0.3680	0.1193
80.42	0.0805	0.3631	0.1389
89.88	0.0776	0.3602	0.1514
100.13	0.0734	0.3560	0.1706

Table 12
 $K_n = 0.01397$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.06212	0.4068	—
10.20	0.05877	0.4035	0.0204
20.00	0.05539	0.4001	0.0426
30.02	0.05217	0.3969	0.0652
40.10	0.04952	0.3942	0.0846
50.10	0.04729	0.3920	0.1024
60.17	0.04434	0.3890	0.1271
69.97	0.04212	0.3868	0.1468
80.17	0.04024	0.3849	0.1647
90.12	0.03764	0.3823	0.1906
100.00	0.03589	0.3806	0.2093

Table 13
 $K_n = 0.01502$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.03106	0.4068	—
10.23	0.02936	0.4051	0.0224
19.97	0.02743	0.4031	0.0500
29.93	0.02581	0.4015	0.0748
40.07	0.02425	0.4000	0.1000
49.97	0.02282	0.3985	0.1248
59.97	0.02150	0.3972	0.1492
70.13	0.02032	0.3960	0.1726
79.95	0.01908	0.3948	0.1987
90.42	0.01819	0.3939	0.2183
99.93	0.01718	0.3929	0.2423

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Table 14
 $K_n = 0.01533$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.01365	0.4047	—
9.90	0.01290	0.4039	0.0237
19.88	0.01202	0.4031	0.0535
29.90	0.01132	0.4024	0.0788
39.93	0.01059	0.4016	0.1069
49.83	0.00995	0.4010	0.1332
59.78	0.00937	0.4004	0.1587
69.97	0.00883	0.3999	0.1841
79.92	0.00833	0.3994	0.2087
89.95	0.00787	0.3989	0.2330
99.95	0.00743	0.3985	0.2574

Table 15
 $K_n = 0.01648$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.006825	0.4047	—
10.10	0.006409	0.4043	0.0269
20.02	0.005980	0.4039	0.0565
30.02	0.005551	0.4034	0.0885
40.02	0.005230	0.4031	0.1139
50.02	0.004850	0.4027	0.1461
59.98	0.004546	0.4024	0.1741
70.00	0.004283	0.4022	0.1998
80.08	0.003986	0.4019	0.2305
90.00	0.003779	0.4017	0.2536
100.13	0.003494	0.4014	0.2871

Table 16

c	Λ	K_n
0.2500	0.879	0.01332
0.1250	1.095	0.01355
0.0625	1.397	0.01397
0.03125	1.790	0.01502
0.01563	2.471	0.01533
0.007813	3.529	0.01648

Tables 17-22. The reaction $(\text{CH}_3\text{COCHCOOC}_2\text{H}_5)\text{K} + \text{CH}_3\text{I}$.

Table 17
 $K_n = 0.1059$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.2540	0.4045	—
5.13	0.2033	0.3538	0.0385
9.87	0.1713	0.3218	0.0717
15.05	0.1437	0.2942	0.1090
19.95	0.1221	0.2726	0.1467
25.07	0.1075	0.2580	0.1781
30.13	0.0937	0.2442	0.2139
35.05	0.0844	0.2349	0.2424
40.07	0.0740	0.2245	0.2799
44.92	0.0648	0.2153	0.3194

Table 18
 $K_n = 0.1070$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.1270	0.4045	—
4.88	0.1014	0.3789	0.0693
9.92	0.0846	0.3621	0.1284
15.00	0.0686	0.3461	0.1998
20.03	0.0586	0.3361	0.2555
24.97	0.0486	0.3261	0.3237
29.88	0.0407	0.3182	0.3901
34.98	0.0344	0.3119	0.4544
39.97	0.0294	0.3069	0.5155

Table 19

 $K_n = 0.1082$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.0604	0.4012	—
5.10	0.0481	0.3889	0.0853
10.08	0.0391	0.3799	0.1653
15.08	0.0320	0.3728	0.2440
20.00	0.0261	0.3667	0.3253
25.12	0.0218	0.3626	0.3986
30.12	0.0178	0.3586	0.4819
35.32	0.0146	0.3554	0.5641
40.42	0.0119	0.3527	0.6495
45.03	0.0103	0.3511	0.7103

Table 20

 $K_n = 0.1081$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.0635	0.4045	—
5.50	0.0491	0.3901	0.0958
10.05	0.0407	0.3817	0.1676
15.08	0.0331	0.3741	0.2490
20.12	0.0270	0.3680	0.3310
25.03	0.0222	0.3632	0.4098
28.00	0.0197	0.3607	0.4588
30.92	0.0177	0.3587	0.5020
34.97	0.0152	0.3562	0.5645
37.92	0.0134	0.3544	0.6178

Table 21

 $K_n = 0.1097$

$t_{\min}$	a	b	$\log \frac{b a_0}{a b_0}$
0	0.03115	0.4025	—
4.08	0.02642	0.3977	0.0663
7.95	0.02236	0.3937	0.1345
11.95	0.01882	0.3901	0.2052
15.92	0.01592	0.3872	0.2746
20.02	0.01325	0.3846	0.3514
23.88	0.01135	0.3827	0.4165
28.15	0.00935	0.3807	0.4985
32.02	0.00801	0.3793	0.5641

Table 22

 $K_i = 0.120, K_n = 0.102.$

c	α	K_n		$K_n/(1-\alpha)$	$\alpha/(1-\alpha)$
		found	calc.		
0.2500	0.2137	0.1058	0.1058	0.1347	0.2718
0.1250	0.2673	0.1070	0.1068	0.1460	0.3648
0.0625	0.3423	0.1082	0.1082	0.1645	0.5204
0.03125	0.4170	0.1097	0.1095	0.1882	0.7152

The velocity constants K_n were measured for the reaction between the potassium compound of ethyl acetoacetate and methyl iodide at 4 different initial concentrations of the potassium compound varying from $M/4$ to $M/32$ (Tables 17–21). At concentrations lower than $M/32$ no reproducible values could be obtained due to the saponification reaction. This side reaction also prevents an exact measurement of the limiting conductivity. However, for concentrations varying from $M/4$ to $M/32$ reproducible values of the conductivity could be obtained which were in fair

agreement with the values obtained by White. Since the limiting conductivity is the same for the sodium compound of ethyl acetoacetate as that for sodium ethoxide the limiting conductivity should be the same for the potassium compound of ethyl acetoacetate as that for potassium ethoxide, viz. $\Lambda_0 = 46.6$. For the potassium compound of ethyl acetoacetate the degree of dissociation can thus be calculated. A plot of $K_n/(1 - \alpha)$ against $\alpha/(1 - \alpha)$ gives a straight line from which the velocity constant can be calculated for the free ethyl acetoacetate anion $K_i = 0.120$ and that for the (chelated) ion pair $K_m = 0.102$ (Table 22, Fig. 2). The value of $K_i = 0.120$ for the potassium compound is close to that for the sodium compound $K_i = 0.117$.

The results obtained are in good agreement with the mechanism given by Brändström (1, 2). When the alkylation of the sodium compound of ethyl acetoacetate is used for preparative purposes the concentration of the sodium compound is usually higher than 1 molar. In such a solution the degree of dissociation is very low (a few per cent) and the alkylation will then proceed as an almost pure chelate reaction (1).

Experimental

Dry alcohol and the ethoxide solutions were prepared by the method given by Brändström (5).

The ethyl acetoacetate and the methyl iodide were pure commercial products purified by distillation.

The kinetic measurements were made at 25°C in a thermostat filled with di-ethylene glycol at days when the humidity was very low in the laboratory.

The reaction solutions were prepared by mixing alcoholic solutions of alkali ethoxides, ethyl acetoacetate and methyl iodide in that order. The time was taken when the methyl iodide had been added.

The reaction was followed by taking aliquots from the reaction mixture by means of a micropipette, and quickly blowing them into an excess of standard hydrochloric acid. The acid excess was then titrated by means of 0.1 *M* sodium hydroxide using methyl red as an indicator or by means of 0.01 *M* sodium tetraborate with phenolphthaline as an indicator. These titrations were performed with a Agla syringe.

SUMMARY

The velocity of the methylation has been measured for alkali compounds of ethyl acetoacetates in pure alcohol.

The reaction was found to proceed both with the free ethyl acetoacetate anion and with the chelated ion-pair. In the concentrated alcoholic solutions used for preparative purposes the reaction between methyl iodide and the sodium compound of ethyl acetoacetate is an almost pure chelate reaction.

The author is indebted to Sven Olov Lind for good help with the manual work.

Research Laboratory of Pharmacia Ltd., Uppsala, February 1958.

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On the crystal structure of a zirconium hydroxide chromate

By GEORG LUNDGREN

With 8 figures in the text

A number of zirconium oxide chromates are described in Gmelin's *Handbuch* (1). They are said to be yellow or brownish yellow powders, some of which lose chromate ions when they are washed with water. This suggests that the compounds described are amorphous and this was verified by repeating the experiments and taking X-ray powder photographs of the products.

In 1929, however, Briggs published a paper (2), where he described the preparation of a crystalline zirconium oxide chromate, to which he ascribed the formula $9\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 12\text{H}_2\text{O}$. First he obtained an amorphous product by precipitation from a zirconium nitrate solution with potassium dichromate and then, after washing and drying it, he digested it with a concentrated aqueous solution of CrO_3 and heated the mixture for $3\frac{1}{2}$ hours at 190°C , when crystals formed.

By repeating and modifying Briggs' experiments it was possible to prepare crystals of zirconium hydroxide chromates large enough for X-ray single crystal investigations. The formulae of the compounds obtained are, however, quite different from that given by Briggs: $4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$ and $\text{ZrO}_2 \cdot \text{CrO}_3 \cdot \text{H}_2\text{O}$ as compared with $9\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 12\text{H}_2\text{O}$. This obviously depends on the short heating time used by Briggs (only $3\frac{1}{2}$ hours). The reaction between the amorphous product and the solution must have been incomplete and his analyses were made on products which still contained amorphous material.

The present paper deals only with the structure of $4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$. The crystal structure determination of $\text{ZrO}_2 \cdot \text{CrO}_3 \cdot \text{H}_2\text{O}$ will be published later.

Preparation of $4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$

The synthesis of the crystals was performed in two steps:

First an amorphous product was prepared: 10 g of potassium dichromate, dissolved in 80 ml of water, were added to a solution of 7 g of commercial zirconium nitrate (Schering A.G., Berlin) in 50 ml of water. A precipitate formed very slowly (one hour after the mixing the solution was still fairly clear). After 1–2 days the precipitate was filtered off, washed with water and dried in air. The product obtained was amorphous to X-rays and had an approximate composition of 50 % ZrO_2 , 21 % CrO_3 , and 29 % H_2O .

The amorphous product prepared was then converted to crystals of $4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$ in the following way: A mixture of 2 g of the amorphous material,

4 g of chromium trioxide (Baker's Analyzed) and 2 ml of water was sealed in a thick-walled Pyrex tube and heated in an oven at 85–190°C. After a week the tube was cooled and opened and the crystals were filtered off, washed with water several times and dried in air. When examined under a microscope, they proved to be small, somewhat elongated, red octahedra, at most 0.2 mm in diameter.

Analysis

A sample, dried in air, was decomposed by being boiled with 20 ml of a sodium hydroxide solution (20 per cent by weight). After dilution with water the solution was filtered. The filtrate, which contained all the chromium as chromate ions, was acidified, 2 grams of potassium iodide were added and the liberated iodine titrated with standard sodium thiosulfate. The amount of *chromium trioxide* in the crystals was then calculated.

The amount of *zirconium dioxide* was determined by igniting a sample and weighing the residue of $\text{ZrO}_2 + \text{Cr}_2\text{O}_3$. This method of analysis tends to give high ZrO_2 values, since the chromium atoms in the oxide mixture incline to retain an average valence slightly higher than three.

The *water* was determined with Penfield's method as described by Kolthoff and Sandell (3).

The *density* was determined from the loss of weight in benzene.

	Found	Calculated for $4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$
% CrO_3	45.8	46.17
% ZrO_2	46.5	45.51
% H_2O	8.2	8.32
Density	3.22 ₅	3.294 (cf. below)

X-ray diffraction data

The crystals obtained were mostly twinned but the samples also contained a few crystals that only had small satellites. They were, however, rather deformed. Some of them were set in a Weissenberg camera and rotation (around [100], [010], and [001]) and Weissenberg photographs ($0kl$ – $2kl$, $h0l$ – $h4l$, $hk0$ – $hk2$) were taken using Cu K radiation and the multiple film technique. The photographs showed that the crystals have the orthorhombic symmetry and that their unit cell dimensions are $a \approx 11.7$ Å, $b \approx 13.7$ Å, $c \approx 6.9$ Å. More accurate values for the cell dimensions were obtained from powder photographs taken in Phragmén focusing cameras using Cr K radiation ($\lambda_{\text{Cr } K\alpha} = 2.2909$ Å) (see Table 1):

$$\begin{array}{ll} a = (11.63 \pm 0.01) \text{ Å} & c = (6.878 \pm 0.005) \text{ Å} \\ b = (13.65 \pm 0.01) \text{ Å} & V = 1092 \text{ Å}^3 \end{array}$$

The unit cell contains two formula units ($4\text{ZrO}_2 \cdot 5\text{CrO}_3 \cdot 5\text{H}_2\text{O}$) giving a calculated density of 3.294 g/ml as compared to the observed value of 3.22₅ g/ml (see above).

The following reflections are systematically absent

$$\begin{array}{l} h0l \text{ with } h + l = \text{odd} \\ 0kl \text{ with } k + l = \text{odd} \end{array}$$

Table 1. Powder photographs of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$. Phragmén cameras.
CrK radiation.

hkl	$10^4 \sin^2 \theta$ calc	$10^4 \sin^2 \theta$ obs	I obs	hkl	$10^4 \sin^2 \theta$ calc	$10^4 \sin^2 \theta$ obs	I obs
0 0 2	1109	1099	m	1 4 3	3719		
0 4 0	1126	1117	st	0 7 1	3726		
3 0 1	1150	1147	st	1 6 2	3740	(3729)	(w)
3 2 0	1155			3 5 2	3742		
3 1 1	1220	1214	vw	4 4 2	3787	3802	vw ⁻
1 2 2	1488	1480	vw	5 2 2	3816		
2 0 2	1497	1494	w	1 7 1	3823	3826	w
1 4 1	1500			5 4 1	3828		
2 4 0	1514	1508	m	2 7 0	3837		
4 0 0	1552	1548	vw ^w	6 1 1	3839		
2 2 2	1779	1777	vw ^w	6 2 1	4051	(4054)	(m)
4 2 1	2111	2109	m	2 7 1	4114		
0 4 2	2235	(2233)	(vw)	4 1 3	4118	(4117)	(vw ^w)
3 2 2	2264	2276	w	4 2 3	4330		
3 4 1	2276			4 6 1	4363	4323	w
				0 0 4	4438	4362	m
						4443	w
1 0 3	2593	2590	w	3 4 3	4495		
2 4 2	2623	2630	m	0 8 0	4505	4507	m
1 6 0	2631			3 6 2	4516		
3 5 0	2633			6 4 0	4618	(4617)	vw ⁻
4 0 2	2661	2674	w	1 2 4	4817		
4 4 0	2678			2 0 4	4826	4824	vw ^w
5 0 1	2702	2705	m	1 8 1	4879		
5 2 0	2707			2 8 0	4893	4880	w ⁺
3 5 1	2910	2902	vw ^w	2 1 4	4896		
2 6 1	3199	3199	w	5 6 0	4959	4963	w
3 0 3	3369	(3363)	(vw)	7 0 1	5030		
3 6 0	3407	3406	st	7 2 0	5035	5034	w ⁻
6 0 0	3492	3500	vw ^w				
1 7 0	3546	3553	vw ^w				

The horizontal line marks the boundary between the angular ranges of the two different focusing cameras used.

Only reflections, which appear both in Weissenberg and powder photographs have been included in the list of $\sin^2 \theta_{\text{calc}}$.

Reflections coinciding with β reflections are given in brackets.

The powder photographs were measured and interpreted to $\sin^2 \theta = 0.70$.

This is characteristic for the two space groups no. 58 $Pn\bar{n}m$ and no. 34 $Pnn2$ in the International Tables (4). The first of them is, however, not probable if the atoms are not arranged statistically in some way or other. We observe, namely, that the unit cell contains ten chromate groups, of which at least two should be situated in a twofold position. This is not possible for $Pn\bar{n}m$ since all twofold positions in this space group are situated at centres of symmetry which does not fit in with a chromate group. The calculations were thus at first performed assuming space group no. 34 $Pnn2$.

Positions of the zirconium atoms

To determine the parameters of the zirconium and chromium atoms the three Patterson projections $P(uvp)$, $P(upw)$, and $P(pvw)$ were calculated, the intensities

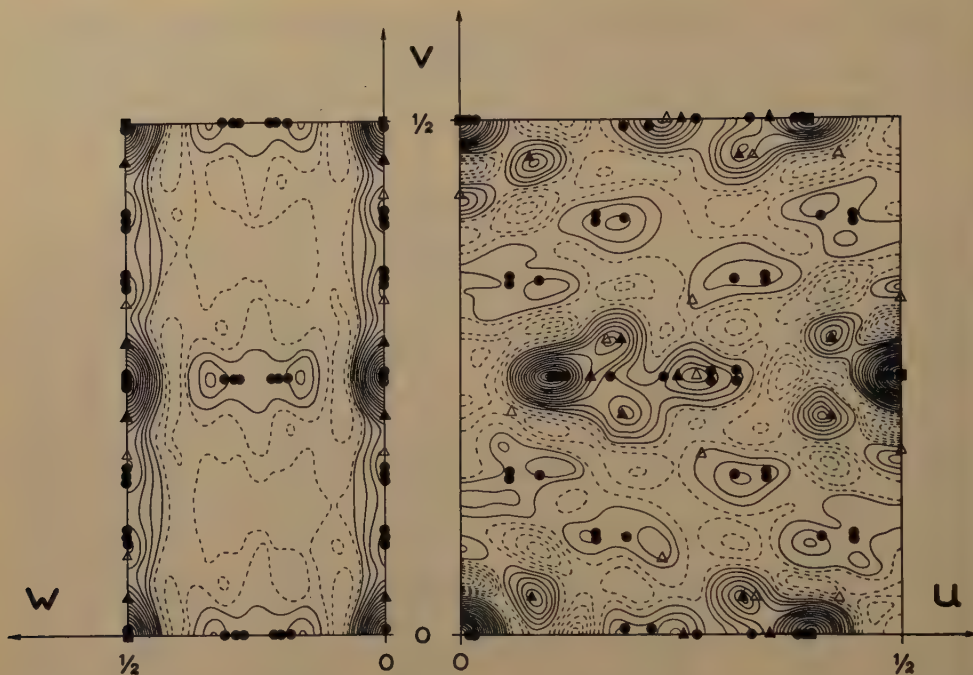


Fig. 1. The Patterson functions $P(uvp)$ (right) and $P(pvw)$ (left). The vectors Zr-Zr (■), Zr-Cr (▲), Zr-O (●), and Cr-Cr (△) for the final structure have been marked. For the maxima $(0, 0)$, $(\frac{1}{2}, \frac{1}{4})$, and $(0, \frac{1}{2})$ in $P(uvp)$ only every second contour has been drawn. Dashed lines indicate negative values.

being estimated visually and corrected according to Lu (5). They are shown in Figs. 1 and 2.

The interatomic vectors within a point position in $Pnn2$ are:

I. $\pm 2x, \pm 2y, 0$	weight 1
II. $\pm (\frac{1}{2} - 2x), \frac{1}{2}, \frac{1}{2}$	„ 2
III. $\frac{1}{2}, \pm (\frac{1}{2} - 2y), \frac{1}{2}$	„ 2

By comparing this table with $P(uvp)$ in Fig. 1 we see at once from the high maximum at $u = \frac{1}{2}, v = \frac{1}{4}$ that the eight zirconium atoms in the unit cell should belong to two fourfold positions 4(c) having $y \approx (2n + 1) \cdot \frac{1}{8}$ ($n = \text{integer}$). The zirconium atoms are thus situated in the following way:

$$\begin{aligned}
 4\text{Zr}_1 \text{ in } Pnn2:4(c) & \text{ with } x = x_1, y \approx (2n_1 + 1) \cdot \frac{1}{8}, z = z_1 \\
 4\text{Zr}_2 \text{ in } Pnn2:4(c) & \text{ with } x = x_2, y \approx (2n_2 + 1) \cdot \frac{1}{8}, z = z_2
 \end{aligned}$$

The distances $\text{Zr}_1\text{-Zr}_2$ would then be:

$$\begin{array}{lll}
 \text{A. } u = \pm (x_1 - x_2), & v \approx \pm (n_1 - n_2) \cdot \frac{1}{4}, & w = \pm (z_1 - z_2) \\
 \text{B. } u = \pm (x_1 + x_2), & v \approx \pm (n_1 + n_2 + 1) \cdot \frac{1}{4}, & w = \pm (z_1 - z_2) \\
 \text{C. } u = \pm (x_1 + x_2 + \frac{1}{2}), & v \approx \pm (n_1 - n_2) \cdot \frac{1}{4} + \frac{1}{2}, & w = \pm (z_1 - z_2 + \frac{1}{2}) \\
 \text{D. } u = \pm (x_1 - x_2 + \frac{1}{2}), & v \approx \pm (n_1 + n_2 + 1) \cdot \frac{1}{4} + \frac{1}{2}, & w = \pm (z_1 - z_2 + \frac{1}{2})
 \end{array}$$

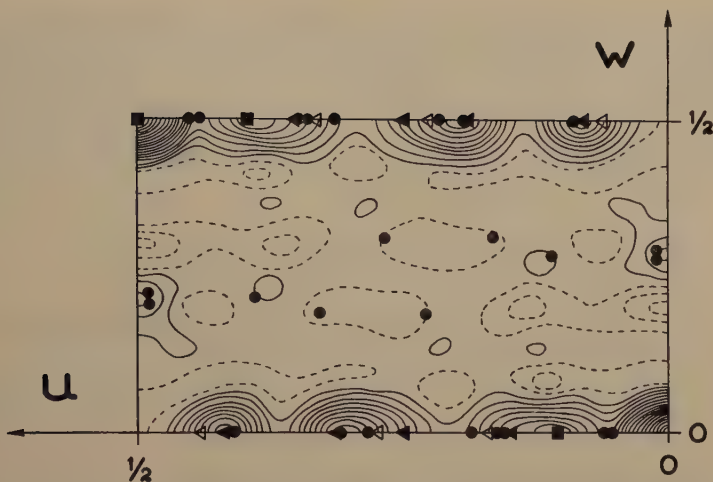


Fig. 2. The Patterson function $P(upw)$. The vectors Zr-Zr (■), Zr-Cr (▲), Zr-O (●), and Cr-Cr (△) for the final structure have been marked. Dashed lines indicate negative values.

By comparing this with $P(pvw)$ and $P(upw)$ in Figs. 1 and 2 we see that the w parameters of A-D are 0 or $\frac{1}{2}$, i.e. $z_1 \approx z_2 \approx 0$, since z_1 can arbitrarily be chosen as 0.

To determine the values of x_1 , x_2 , n_1 , and n_2 the generalized Patterson function $P_1(uv) = \sum_h \sum_k |F_{hkl}|^2 \cos 2\pi(hu + kv)$ was calculated (see Fig. 3, left). All Zr-Zr maxima with $w = 0$ should appear as large positive peaks in this function, whereas

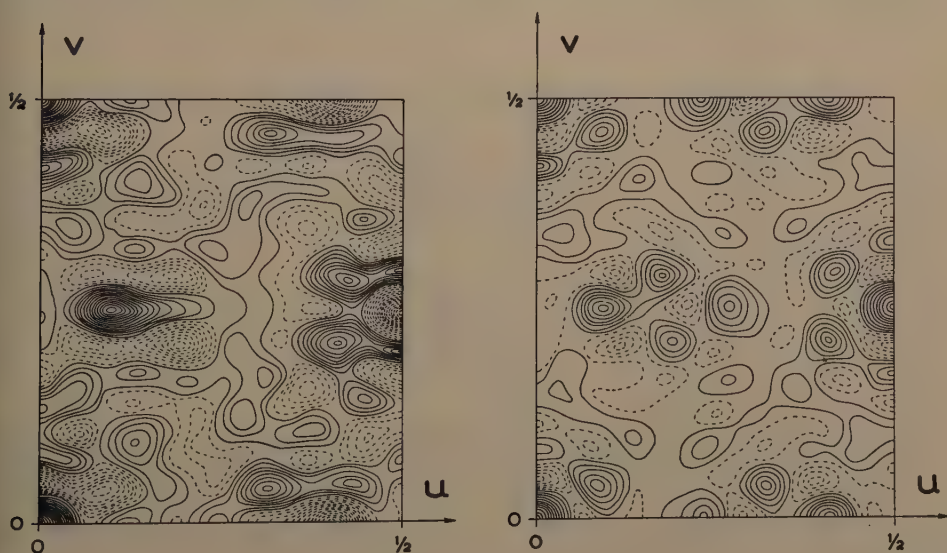


Fig. 3. The generalized Patterson functions $P_1(uv)$ (left) and $P_2(uv)$ (right). In the maxima or minima $(0, 0)$, $(\frac{1}{2}, \frac{1}{2})$, and $(0, \frac{1}{2})$ only every second contour has been drawn. Dashed lines indicate negative values.

all maxima with $w = \frac{1}{2}$ should appear as deep negative pits. By comparing the vectors I–III with Fig. 3 we see that vector II should be situated at $(\pm 0.396, \frac{1}{2}, \frac{1}{2})$ for both Zr_1 and Zr_2 . The possible values of x_1 and x_2 should thus be $\pm 0.05_2$ or $\pm 0.44_8$. By calculating the vectors A–D for all possible combinations of x_1 , x_2 , n_1 , and n_2 and comparing the values obtained with Figs. 1, 2, and 3 the following arrangement was found for the zirconium atoms (the space considered could be restricted to $0 \leq x_1 \leq \frac{1}{4}$, $0 \leq x_2 \leq \frac{1}{2}$, $n_1 = 0$, $n_2 = 0, 1, 2, 3$):

$$\begin{aligned} 4Zr_1 \text{ in } Pnn2:4(c) & \text{ with } x \approx 0.05_2, y \approx 0.12_5, z \approx 0 \\ 4Zr_2 \text{ in } Pnn2:4(c) & \text{ with } x \approx 0.05_2, y \approx 0.62_5, z \approx 0 \end{aligned}$$

The zirconium atoms thus form zigzag chains which run through the lattice in the b direction. The shortest distances Zr–Zr within the chains are $3.6_2 \text{ \AA}$.

Positions of the chromium atoms

The ten chromium atoms in the unit cell should be arranged in one of the following ways:

1. $4Cr_1$ in 4(c), $4Cr_2$ in 4(c), and $2Cr_3$ in 2(a) or 2(b)
2. $4Cr_1$ in 4(c), six chromium atoms in twofold positions
3. All ten chromium atoms in twofold positions

The two latter alternatives should give Zr–Cr_{twofold} vectors of high multiplicity at $(\pm 0.05_2, \pm 0.12_5)$, $(\pm 0.05_2, \pm 0.37_5)$, $(\pm 0.44_8, \pm 0.12_5)$ and $(\pm 0.44_8, \pm 0.37_5)$ in $P(uvp)$. By comparison with Fig. 1 we see, however, that the actual maxima at these points are very low, whereas much higher maxima are still unexplained. The arrangements 2 and 3 can thus be ruled out and the chromium atoms should be situated as given by 1.

Parameters of the fourfold chromium positions Cr_1 and Cr_2

The z parameters of the two fourfold chromium positions Cr_1 and Cr_2 should certainly be ≈ 0 , since both Patterson projections $P(pvw)$ and $P(upw)$ (see Figs. 1 and 2) show only weak maxima outside the lines $w = 0$ and $w = \frac{1}{2}$. The vectors Zr–Cr_{fourfold} would then be:

$$\begin{array}{lll} u = \pm(x_{Zr} - x_{Cr}) & v = \pm(y_{Zr} - y_{Cr}) & w = 0 \\ u = \pm(x_{Zr} + x_{Cr}) & v = \pm(y_{Zr} + y_{Cr}) & w = 0 \\ u = \pm(\frac{1}{2} + x_{Zr} - x_{Cr}) & v = \pm(\frac{1}{2} + y_{Zr} + y_{Cr}) & w = \frac{1}{2} \\ u = \pm(\frac{1}{2} + x_{Zr} + x_{Cr}) & v = \pm(\frac{1}{2} + y_{Zr} - y_{Cr}) & w = \frac{1}{2} \end{array}$$

The vectors have all the same weight.

By identifying the first vector with those distinct peaks in $P(uvp)$ (Fig. 1) that have $w = 0$ according to $P_1(uv)$ in Fig. 3, left, a number of alternatives for x_{Cr} and y_{Cr} were obtained. They were then tested with the other Zr–Cr vectors above, when only the following possible sets of parameters were left for fourfold chromium positions:

$$\begin{array}{llll} \text{(a)} & x_{Cr} \approx 0.37 & \text{(b)} & x_{Cr} \approx 0.37 \\ & y_{Cr} \approx 0.09 & & y_{Cr} \approx 0.16 \\ & z_{Cr} \approx 0 & & z_{Cr} \approx 0 \\ \text{(c)} & x_{Cr} \approx 0.37 & \text{(d)} & x_{Cr} \approx 0.37 \\ & y_{Cr} \approx 0.59 & & y_{Cr} \approx 0.66 \\ & z_{Cr} \approx 0 & & z_{Cr} \approx 0 \end{array}$$

All these possibilities give the Zr-Cr vectors indicated in Figs. 1 and 2.

However, only two of the positions (a)-(d) are occupied by chromium atoms. We see then at once that (a) cannot be combined with (b) nor (c) with (d) since some of the distances Cr-Cr are too short (≈ 1 Å). Further, the atomic arrangement given by (a) + (c) is the same as that given by (b) + (d). In the same way (a) + (d) is identical with (b) + (c). The following two possibilities are thus arrived at for the eight chromium atoms arranged in fourfold positions:

- A. 4Cr_1 in $Pnn2:4(c)$ with $x \approx 0.37$, $y \approx 0.09$, $z \approx 0$
 4Cr_2 in $Pnn2:4(c)$ with $x \approx 0.37$, $y \approx 0.59$, $z \approx 0$
 B. 4Cr_1 in $Pnn2:4(c)$ with $x \approx 0.37$, $y \approx 0.09$, $z \approx 0$
 4Cr_2 in $Pnn2:4(c)$ with $x \approx 0.37$, $y \approx 0.66$, $z \approx 0$

By calculating the structure factors due to the Zr_1 , Zr_2 , Cr_1 , and Cr_2 atoms we find that A gives the special extinction hkl absent when k is odd, whereas B gives hkl absent when

$$\begin{cases} h+l=2n \\ k=4m+2 \end{cases} \text{ and } \begin{cases} h+l=2n+1 \\ k=4m \end{cases}$$

Now, we could detect many reflections with $k = \text{odd}$ in the photographs but no reflections satisfying the latter conditions. We could thus conclude that alternative B should give the correct chromium arrangement for the two fourfold positions Cr_1 and Cr_2 .

The chromium atoms Cr_3

General considerations

The remaining two chromium atoms (Cr_3) should, according to $Pnn2$, be situated at $(0,0,z)$, $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2} + z)$ or at $(0, \frac{1}{2}, z)$, $(\frac{1}{2}, 0, \frac{1}{2} + z)$, giving Zr- Cr_3 vectors at $(\pm 0.05_2, \pm 0.12_5)$; $(\pm 0.05_2, \pm 0.37_5)$; $(\pm 0.44_8, \pm 0.12_5)$; and $(\pm 0.44_8, \pm 0.37_5)$ in $P(uvp)$. By comparison with Fig. 1 we see, however, that there are no definite maxima at these points, though they are situated in very low, positive regions. This fact suggests that the Cr_3 atoms are arranged in some way other than that given by $Pnn2$. We therefore investigated if an arrangement for the Zr_1 , Zr_2 , Cr_1 , Cr_2 , and Cr_3 atoms could be obtained through special positions in some space group of lower symmetry, which generally has only part of the systematic extinctions given above on page 60. The Laue symmetry was still considered to be mmm since no deviations from the orthorhombic symmetry could be detected in any of the Weissenberg photographs.

The following survey gives a summary of the results with these considerations:

- (a) Space groups no. 59 $Pmmm$,¹ no. 31 $Pmn2_1$, no. 47 $Pmmm$, no. 25 in the two orientations $Pmm2$ and $Pm2m$,¹ no. 18 $P2_122_1$,¹ no. 17 $P22_12$,¹ and no. 16 $P222$.

In these space groups it was not possible to find any set of zirconium parameters which was in agreement with the Patterson functions $P(uvp)$, $P(upw)$, $P(pvw)$, $P_1(uv)$, and $P_2(uv)$.

- (b) Space group no. 19 $P2_12_12_1$.

This space group has only fourfold positions and consequently it could not explain the arrangement of the ten chromate groups in the unit cell.

¹ Orientation, different from that given in the International Tables (4).

- (c) Space groups no. 59 $Pnmm$, no. 31 $Pnm2_1$,¹ no. 25 $P2mm$,¹ no. 18 $P2_12_1$,¹ and no. 17 $P222_1$.

With these space groups it was possible to determine zirconium positions giving Zr-Zr vectors that fitted in with all the Patterson functions calculated. The arrangements found were the same as given above for $Pnn2$. However, when trying to determine chromium (Cr_1 and Cr_2) parameters we could either not get sufficient agreement between the calculated Zr-Cr vectors and the observed maxima in $P(uvp)$, $P(upw)$, $P(pvw)$, $P_1(uv)$, and $P_2(uv)$, or, when a rough agreement was obtained, get too short Cr-Cr distances (only about 1 Å).

- (d) Space groups no. 31 in the two orientations $P2_1nm$ and $Pn2_1m$,¹ no. 18 $P2_12_1$,² and no. 17 $P2_122_1$.¹

With these space groups it was possible to find a plausible set of parameters for the zirconium and eight of the ten chromium atoms in the unit cell. The atomic arrangement found was the same as that given above for Zr_1 , Zr_2 , Cr_1 , and Cr_2 in $Pnn2$.

The remaining two chromium atoms (Cr_3) ought to be arranged in twofold positions. They should, however, not be situated on the lines $(0, 0, z)$, $(0, \frac{1}{2}, z)$, $(\frac{1}{2}, 0, z)$, or $(\frac{1}{2}, \frac{1}{2}, z)$ since the corresponding Zr- Cr_3 maxima are not in agreement with $P(uvp)$ (see above for $Pnn2$). The only positions to be considered then should be $P2_1nm:2(a)$, $Pn2_1m:2(a)$, and $P2_122:2(c)$ and $2(d)$. The two latter positions can be ruled out immediately, since they would give Zr- Cr_3 maxima at $u = \frac{1}{4} \pm 0.05_2$, $w = \frac{1}{4}$ in $P(upw)$, where there are only negative regions.

By considering all the possible, characteristic and non-characteristic, orthorhombic space groups we have thus found that, if the ten chromium atoms in the unit cell are to be arranged in definite point positions, the correct space group is $P2_1nm$ or $Pn2_1m$. We should, however, not disregard the possibility that the two chromate atoms Cr_3 belong to a statistical arrangement in one of the characteristic space groups $Pnn2$ and $Pnnm$.

Determination of the Cr_3 parameters

To determine the parameters of the two Cr_3 atoms we should locate the Zr- Cr_3 vectors in the Patterson functions assuming that the Cr_3 atoms are arranged in one of the positions $P2_1nm:2(a)$,² or $Pn2_1m:2(a)$.³ The vectors are the same for both cases and also the same as those obtained with $Pnn2:4(c)$, $z = 0$ or with $Pnnm:4(g)$. The vectors are:

$$\begin{aligned} & (0, 0, 0; 0, \tfrac{1}{2}, 0) + \\ & u = \pm (x_{Cr_3} - 0.05_3); \quad v = \pm (y_{Cr_3} - 0.12_5); \quad w = 0 \\ & u = \pm (x_{Cr_3} + 0.05_3); \quad v = \pm (y_{Cr_3} + 0.12_5); \quad w = 0 \\ & u = \pm (x_{Cr_3} - 0.44_8); \quad v = \pm (y_{Cr_3} - 0.62_5); \quad w = \tfrac{1}{2} \\ & u = \pm (x_{Cr_3} + 0.44_8); \quad v = \pm (y_{Cr_3} + 0.62_5); \quad w = \tfrac{1}{2} \end{aligned}$$

¹ Orientation, different from that given in the International Tables (4).

² $P2_1nm:2(a)$: $(x, y, 0)$; $(\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2})$.

³ $Pn2_1m:2(a)$: $(x, y, 0)$; $(\frac{1}{2} - x, \frac{1}{2} + y, \frac{1}{2})$.

Now, by examining the Patterson function $P(uvp)$ in Fig. 1, we see that, in addition to the Zr–Zr, Zr–Cr₁, and Zr–Cr₂ peaks, there are three more maxima that are rather pronounced and that are not probably caused by diffraction effects. Their parameters are $u = \pm 0.241$, $v = 0$; $u = \pm 0.230$, $v = \frac{1}{2}$; and $u = \pm 0.267$, $v = \pm 0.250$. Two of them contain peaks due to Cr₁–Cr₁ and Cr₂–Cr₂ vectors ($= \pm 0.24, \frac{1}{2}, \frac{1}{2}$), or Cr₁–Cr₂ vectors ($= \pm 0.26, \pm 0.25, 0$). They are, however, higher than could be expected for Cr–Cr maxima and they must thus contain some other peaks too.

In $P_1(uv)$ (Fig. 3, left) there is a maximum only at one of these positions ($\pm 0.241, 0$) whereas the two other peaks are missing, the function being close to 0 in these regions. In the generalized Patterson function $P_2(uv) = \sum_h \sum_k |F_{hk2}|^2 \cos 2\pi(hu + kv)$, the three peaks are again visible (at $\pm 0.231, 0$; $\pm 0.231, \frac{1}{2}$; and $\pm 0.269, \pm 0.250$) with about the same relative heights as in $P(uvp)$.

All these facts can best be explained by assuming that the following vectors exist in the unit cell (the values are weighted means of the parameters found in $P(uvp)$, $P_1(uv)$, and $P_2(uv)$):

$$\begin{array}{lll} \text{(a)} & u = \pm 0.23_5 & v = 0 & w = 0 \\ \text{(b)} & u = \pm 0.23_0 & v = \frac{1}{2} & w = 0 \\ \text{(c)} & u = \pm 0.26_8 & v = \pm 0.25_0 & w = \frac{1}{2} \end{array}$$

Since the corresponding maxima in $P(uvp)$ and $P_2(uv)$ are fairly distinct it seems reasonable to consider them as some of the Zr–Cr₃ vectors sought for.

By identifying the vectors (a)–(c) with the calculated Zr–Cr₃ vectors above, a number of possible parameters for the Cr₃ atoms were obtained. They were checked by calculating all Zr–Cr₃ vectors and comparing the results with $P(uvp)$, $P_1(uv)$, and $P_2(uv)$ (Figs. 1 and 3). The following sets of parameters were found to be in agreement with the functions:

$$\begin{array}{llll} \left\{ \begin{array}{l} x_{\text{Cr}_3} \approx 0.18 \\ y_{\text{Cr}_3} \approx 0.87_5 \end{array} \right. & \left\{ \begin{array}{l} x_{\text{Cr}_3} \approx 0.18 \\ y_{\text{Cr}_3} \approx 0.37_5 \end{array} \right. & \left\{ \begin{array}{l} x_{\text{Cr}_3} \approx 0.82 \\ y_{\text{Cr}_3} \approx 0.12_5 \end{array} \right. & \left\{ \begin{array}{l} x_{\text{Cr}_3} \approx 0.82 \\ y_{\text{Cr}_3} \approx 0.62_5 \end{array} \right. \end{array}$$

Owing to the symmetry of the Zr, Cr₁, and Cr₂ arrangements the two latter alternatives are the same as the two former (they can be transformed into each other by merely changing the directions of the x and y axes).

Attempts were then made to decide which is the correct possibility by calculating the Cr₁–Cr₃ and Cr₂–Cr₃ vectors. The results were, however, not conclusive even if the second alternative seemed to be in somewhat better agreement with the calculated functions than the first one. (For both alternatives some of the maxima fell on Zr–Cr₁ or Zr–Cr₂ peaks in $P(uvp)$ and the others in the negative diffraction rings surrounding the high Zr–Zr maxima. The peaks due to Cr₁–Cr₃ or Cr₂–Cr₃ vectors should, however, be very weak and should not appear clearly in the Patterson functions.)

The determination of the correct Cr₃ arrangement had therefore to be postponed until some details of the electron density in the unit cell were known.

Determination of the oxygen parameters

Parameters from $P(upw)$ and $P(pvw)$

Since the arrangement of the Zr₁, Zr₂, Cr₁, and Cr₂ atoms certainly belongs to the symmetry given by space group $Pnn2$ (or $Pnnm$), the determination of the oxygen

positions was performed at first assuming that most of the oxygen arrangement also has this symmetry. A suitable way to start the investigation in order to find the oxygen parameters is to locate the oxygen atoms with $z \neq 0$ and $\frac{1}{2}$, and to consider those maxima in $P(upw)$ and $P(pvw)$ that have $w \neq 0$ and $\frac{1}{2}$.

The distances between the zirconium atoms and oxygen atoms situated in a position 4(c) of $Pnn2$ are:

In $P(upw)$:		In $P(pvw)$:	
$(0, 0; \frac{1}{2}, \frac{1}{2}) +$		$(0, 0; \frac{1}{2}, \frac{1}{2}) +$	
1. $u = \pm(x_0 - 0.05_5)$	$w = \pm z_0$	1. $v = \pm(y_0 - 0.12_5)$	$w = \pm z_0$
2. $u = \pm(x_0 + 0.05_5)$	$w = \pm z_0$	2. $v = \pm(y_0 + 0.12_5)$	$w = \pm z_0$
		3. $v = \pm(y_0 - 0.62_5)$	$w = \pm z_0$
		4. $v = \pm(y_0 + 0.62_5)$	$w = \pm z_0$

Now, all the maxima to be considered in $P(pvw)$ have $v = 0, \frac{1}{4}, \frac{1}{2}$ or $\frac{3}{4}$ (cf. Fig. 1). This means that $y_0 \approx 0.12_5, 0.37_5, 0.62_5$, or 0.87_5 , i.e. the oxygen atoms in question have the same y parameters as the zirconium atoms.

The x_0 and z_0 parameters are both most easily determined from $P(upw)$, since this function is based on a larger number of observed reflections than $P(pvw)$ and consequently should be less influenced by diffraction effects.

In $P(upw)$ (Fig. 2) there are two distinct Zr-O maxima with $w \neq 0$ or $\frac{1}{2}$, one at $u = 0, w = \pm 0.28_7$ and one at $u = \frac{1}{2}, w = \pm 0.21_3$. By comparing these with the calculated vectors above, we see that the following values are possible for x_0 and z_0 (we need only consider the ranges $0 \leq x_0 \leq \frac{1}{2}, 0 \leq z_0 \leq \frac{1}{2}$, if $0 \leq y_0 \leq 1$):

$$\begin{cases} x_0 \approx 0.05 \\ z_0 \approx 0.29 \end{cases} \quad \begin{cases} x_0 \approx 0.45 \\ z_0 \approx 0.21 \end{cases}$$

These two alternatives were combined with the four possible values of y_0 and the parameter sets obtained in this way were tested by comparing all the calculated Zr-O vectors with $P(upw)$, $P(pvw)$, $P(uvp)$, $P_1(uv)$, and $P_2(uv)$ (Figs. 1, 2, and 3). Only the following four combinations were found to be in agreement with the functions:

$$\begin{cases} x_0 \approx 0.05 \\ y_0 \approx 0.12_5 \\ z_0 \approx 0.29 \end{cases} \quad \begin{cases} x_0 \approx 0.05 \\ y_0 \approx 0.62_5 \\ z_0 \approx 0.29 \end{cases} \quad \begin{cases} x_0 \approx 0.45 \\ y_0 \approx 0.12_5 \\ z_0 \approx 0.21 \end{cases} \quad \begin{cases} x_0 \approx 0.45 \\ y_0 \approx 0.62_5 \\ z_0 \approx 0.21 \end{cases}$$

These positions have the same x and y parameters as the zirconium atoms and are thus situated above and below them at a distance Zr-O of 2.0 Å, which is a reasonable contact distance between zirconium and oxygen atoms.

The shortest distances between these oxygen positions and the chromium atoms Cr_1 and Cr_2 are ≈ 1.8 Å. This is fairly close to the Cr-O distances observed within chromate groups (≈ 1.60 Å). Each of the oxygen positions above is thus in close contact with one zirconium atom and one chromium atom. This seems very reasonable, and we conclude that 16 of the 40 chromate oxygen atoms in the unit cell are arranged as follows:

$4O_{1a}$ in $Pnn2:4(c)$	with $x \approx 0.05, y \approx 0.12_5, z \approx 0.29$
$4O_{1b}$ „ „	„ $x \approx 0.45, y \approx 0.62_5, z \approx 0.21$
$4O_{2a}$ „ „	„ $x \approx 0.05, y \approx 0.62_5, z \approx 0.29$
$4O_{2b}$ „ „	„ $x \approx 0.45, y \approx 0.12_5, z \approx 0.21$

Electron density calculations

Both from the general appearance of the Patterson functions $P(pvw)$ and $P(upw)$ (Figs. 1 and 2) and from geometrical reasons, we would expect that most of the remaining oxygen atoms should have their z parameters ≈ 0 or $\frac{1}{2}$. Their positions might then be determined from $P(uvw)$, $P_1(uv)$, and $P_2(uv)$, which would, however, be a rather difficult problem, since there are so many parameters to be determined and many of the Zr-O vectors would overlap each other in the functions mentioned. It would therefore be better to calculate the electron density, even if there is a certain risk that the Cr₃ atoms are arranged without a centre of symmetry. (The Cr₃ atoms give only a small contribution to the intensities and some information on the oxygen arrangement around the centro-symmetrically arranged Zr, Cr₁, and Cr₂ atoms would, in any case, be obtained.)

The electron density projection $\varrho(xyp)$ and the generalized electron density projections $\varrho_1(xy) = \sum_h \sum_k F_{hk1} \cos 2\pi(hx + ky)$ and $\varrho_2(xy) = \sum_h \sum_k F_{hk2} \cos 2\pi(hx + ky)$ were calculated using the F_{hk0} , F_{hk1} , and F_{hk2} values for which the signs could be determined from the known parameters of the Zr, Cr₁, Cr₂, and O_{1a}-O_{2b} atoms. 69 out of 88 observed structure factors $hk0$, 52 out of 63 observed structure factors $hk1$, and 55 out of 64 observed structure factors $hk2$ could then be given a sign.

The results are shown in Fig. 4. We see there that, in addition to the Zr, Cr₁, and Cr₂ peaks, there are some more, quite distinct maxima in $\varrho(xyp)$ and $\varrho_2(xy)$. They have the following parameters (the maxima in the diffraction rings (cf. Doi, 6) around the zirconium atoms have been omitted):

I.	$x \approx 0.20$	$y \approx 0.37_5$	distinct in both $\varrho(xyp)$ and $\varrho_2(xy)$
II.	$x \approx 0.22$	$y \approx 0.13$	distinct, but more or less overlapping each other in both $\varrho(xyp)$
III.	$x \approx 0.28$	$y \approx 0.12$	and $\varrho_2(xy)$
IV.	$x \approx 0.15$	$y \approx 0.28$	distinct in $\varrho(xyp)$, positive region in $\varrho_2(xy)$
V.	$x \approx 0.14$	$y \approx 0.47$	distinct in $\varrho(xyp)$, positive region in $\varrho_2(xy)$
VI.	$x \approx 0.08$	$y \approx 0.28$	distinct in $\varrho_2(xy)$, positive region in $\varrho(xyp)$
VII.	$x \approx 0.08$	$y \approx 0.48$	distinct in $\varrho_2(xy)$, positive region in $\varrho(xyp)$
VIII.	$x \approx 0.40$	$y \approx 0.29$	distinct in $\varrho_2(xy)$, less distinct in $\varrho(xyp)$
IX.	$x \approx 0.41$	$y \approx 0.46$	distinct in $\varrho_2(xy)$, less distinct in $\varrho(xyp)$
X.	$x \approx 0.29$	$y \approx 0.22$	distinct in $\varrho_2(xy)$, extremely weak in $\varrho(xyp)$
XI.	$x \approx 0.20$	$y \approx 0.04$	distinct in $\varrho_2(xy)$, not visible in $\varrho(xyp)$
XII.	$x \approx 0.31$	$y \approx 0.37$	weak maximum in both $\varrho_2(xy)$ and $\varrho(xyp)$

In order to find out which of these maxima are real, and to determine their z parameters (i.e. if $z = 0$ or $\frac{1}{2}$), we study $\varrho_1(xy)$ at the corresponding positions. We see from Fig. 4 that I, VI, and VII correspond to maxima of about the same heights as those in $\varrho(xyp)$ or $\varrho_2(xy)$. The z parameters of I, VI, and VII should thus be ≈ 0 . IV, V, VIII, and IX all correspond to pits in $\varrho_1(xy)$, and the depths of the pits are about the same as the heights of the maxima in $\varrho(xyp)$ or $\varrho_2(xy)$. Their z parameters should therefore be $\approx \frac{1}{2}$.

As for II and III they are situated so close to each other in $\varrho(xyp)$ and $\varrho_2(xy)$ that, if they are both real, one of them must be situated close to the plane $z = 0$, and the other close to the plane $z = \frac{1}{2}$. This is also in agreement with $\varrho_1(xy)$ which, at the II and III positions, shows only a very weak positive region lower than the corresponding maxima in $\varrho(xyp)$ and $\varrho_2(xy)$.

The positions X, XI, and XII are at present left out of the discussions of possible oxygen positions, since for them the results from the examination of $\varrho(xyp)$, $\varrho_1(xy)$,

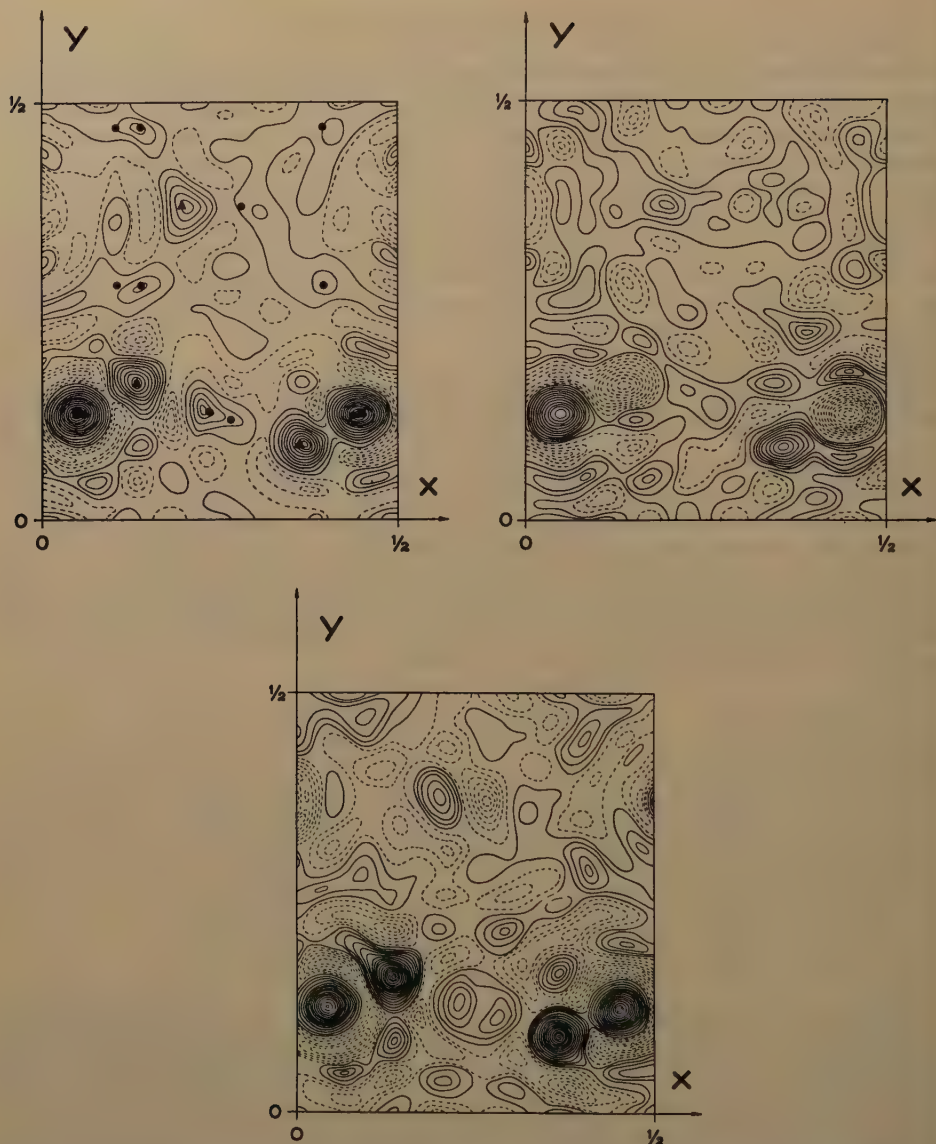


Fig. 4. The preliminary electron density projection $\rho(xyp)$ (upper left) and the preliminary generalized electron density functions $\rho_1(xy)$ (upper right) and $\rho_2(xy)$ (below). For $\rho(xyp)$, the final positions of the zirconium (■), chromium (▲), and oxygen (●) atoms have been marked. In the zirconium maxima or minima, only every second contour has been drawn. Dashed lines indicate negative values.

and $\rho_2(xy)$ are not conclusive. (X is missing in both $\rho_1(xy)$ and $\rho(xyp)$, XI in $\rho(xyp)$, and XII in $\rho_1(xy)$.)

The following possible atomic positions in the unit cell have thus been located:

I.	$x \approx 0.20$	$y \approx 0.37_5$	$z \approx 0$
II.	$x \approx 0.22$	$y \approx 0.13$	$z \approx 0$ or $\frac{1}{2}$
III.	$x \approx 0.28$	$y \approx 0.12$	$z \approx \frac{1}{2}$ or 0
IV.	$x \approx 0.15$	$y \approx 0.28$	$z \approx \frac{1}{2}$
V.	$x \approx 0.14$	$y \approx 0.47$	$z \approx \frac{1}{2}$
VI.	$x \approx 0.08$	$y \approx 0.28$	$z \approx 0$
VII.	$x \approx 0.08$	$y \approx 0.48$	$z \approx 0$
VIII.	$x \approx 0.40$	$y \approx 0.29$	$z \approx \frac{1}{2}$
IX.	$x \approx 0.41$	$y \approx 0.46$	$z \approx \frac{1}{2}$

All these atomic positions are in good agreement with the Patterson functions since all the Zr-X vectors fall in positive regions.

We should now decide if each of the positions above is real or only caused by some diffraction effect. We observe that I coincides with the second of the two alternative Cr₃ positions given above in page 67. The height of its maximum in $\rho(xyp)$, $\rho_1(xy)$, and $\rho_2(xy)$ is about half of the heights of the Cr₁ and Cr₂ maxima. Since there are only weak positive regions at the first alternative Cr₃ position, we conclude that the Cr₃ atoms are situated in the following way:

$$2\text{Cr}_3 \text{ in } P2_1nm:2(a), Pn2_1m:2(a), Pnn2:4(c), \text{ or } Pnnm:4(g) \\ \text{with } x \approx 0.20, y \approx 0.37_5, z \approx 0.$$

Since in this way all zirconium and chromium atoms have been located, the positions II-IX should correspond to oxygen atoms in the unit cell. In order to check that the maxima or minima corresponding to position II-IX are real, we calculated the distances of the oxygen atoms to the zirconium or chromium atoms, for we should expect that most of the oxygen atoms are in close contact with one or two of the metal atoms. We found from the calculations that

(a) the Zr₁ atoms are in close contact ($\approx 2.1 \text{ \AA}$) with the positions II (if $z_{\text{II}} \approx 0$), VI, VIII, and IX (in two directions; one distance however being $2.5 \text{ \AA}$);

(b) the Zr₂ atoms are in close contact ($\approx 2.1\text{--}2.3 \text{ \AA}$) with the positions III (if $z_{\text{III}} \approx \frac{1}{2}$), VI, VII (in two directions), and VIII;

(c) the Cr₁ atoms are in close contact ($\approx 1.7 \text{ \AA}$) with the positions II (if $z_{\text{II}} \approx 0$), and V;

(d) the Cr₂ atoms are in close contact ($\approx 1.7 \text{ \AA}$) with the positions III (if $z_{\text{III}} \approx \frac{1}{2}$) and IV;

(e) the Cr₃ atoms are in contact ($\approx 1.9 \text{ \AA}$) with the positions VI and VII;

(f) the distances Cr₁-position III and Cr₂-position II are too short (only $\approx 1.1 \text{ \AA}$) if $z_{\text{II}} \approx \frac{1}{2}$ and $z_{\text{III}} \approx 0$;

(g) every other distance between the metal atoms and any of the positions II-IX is too long to give a contact.

All this would suggest that II (with $z_{\text{II}} \approx 0$), III (with $z_{\text{III}} \approx \frac{1}{2}$), IV, and V should correspond to the remaining chromate oxygen atoms coordinated to Cr₁ and Cr₂. Each of these chromate groups should then have three of their four oxygen atoms coordinated also to zirconium, which is very plausible. Since the Zr₁, Zr₂, Cr₁, and Cr₂ atoms are all arranged according to space group $Pnn2$ (or $Pnnm$), it is also plausible that the oxygen atoms corresponding to the positions II, III, IV, and V are arranged according to $Pnn2$ (or $Pnnm$). We thus conclude:

4O ₃	in $Pnn2:4(c)$	with $x \approx 0.22, y \approx 0.13, z \approx 0$
4O ₄	„ „ „	$x \approx 0.22, y \approx 0.62, z \approx 0$
4O ₅	„ „ „	$x \approx 0.35, y \approx 0.78, z \approx 0$
4O ₆	„ „ „	$x \approx 0.36, y \approx 0.97, z \approx 0$

Each of the two positions VIII and IX are in two directions coordinated to zirconium. This is very plausible for "basic" oxygen atoms (= hydroxide or oxide ions), and we conclude that VIII and IX are real and give the positions of oxide or hydroxide ions in the unit cell. The symmetry of this arrangement should certainly correspond to that given by the zirconium atoms, i.e. $Pnn2$ (or $Pnnm$), which assumption is also supported by the fact that the VIII and IX maxima or minima in $\rho(xyp)$, $\rho_1(xy)$, and $\rho_2(xy)$ are of the same magnitude as the maxima or minima II-V, due to chromate oxygen atoms belonging to Cr_1 and Cr_2 . The parameters of the oxygen atoms corresponding to VIII and IX should thus be given as follows:

$$\begin{array}{lll} 4O_7 \text{ in } Pnn2:4(c) & \text{with } x \approx 0.10, y \approx 0.79, z \approx 0 \\ 4O_8 \text{ ,, ,, ,, } & \text{,, } x \approx 0.09, y \approx 0.96, z \approx 0 \end{array}$$

The positions VI and VII seem to correspond to two of the oxygen atoms belonging to the Cr_3 atoms. Each of them is also coordinated to two zirconium atoms. Their symmetry should correspond to that of the Cr_3 atoms, i.e. $Pn2_1m$, $P2_1nm$, or, statistically, $Pnn2$ (or $Pnnm$).

However, the maxima or minima VI and VII in $\rho(xyp)$, $\rho_1(xy)$, and $\rho_2(xy)$ are of about the same magnitude as those for the positions II-V, VIII, and IX. Moreover, if each of the positions VI and VII corresponds to only two oxygen atoms, belonging to Cr_3 , the zirconium atoms would be coordinated some to seven, some to six, and some to only five oxygen atoms. The coordination figure around the five-coordinated zirconium atoms would also be very unsymmetrical. It therefore seems much more probable that four of the remaining oxygen atoms (= oxide or hydroxide ions) are situated so as to complete the positions VI and VII to give the symmetry of space group $Pnn2$ (or $Pnnm$). Each of the zirconium atoms would then be coordinated to seven oxygen atoms arranged in an almost regular pentagonal bipyramid.

We thus conclude that the positions VI and VII above correspond to four chromate oxygen atoms and four hydroxide (or oxide) ions arranged as follows:

$$\begin{array}{lll} 4O_9 \text{ in } Pnn2:4(c) & \text{with } x \approx 0.08, y \approx 0.28, z \approx 0 \\ 4O_{10} \text{ ,, ,, ,, } & \text{,, } x \approx 0.08, y \approx 0.48, z \approx 0 \end{array}$$

The positions of the remaining oxygen atoms belonging to the Cr_3 chromate groups can easily be determined geometrically. They were found to be situated close to $x \approx 0.28$, $y \approx 0.375$, $z \approx \pm 0.19$. In the xy projection of the structure this is close to position XII which can thus be explained for $\rho(xyp)$. The symmetry of these oxygen positions should certainly correspond to the symmetry of the Cr_3 atoms.

The positions X and XI (cf. page 69) cannot possibly be occupied by oxygen atoms, since their distances to O_3 and O_4 are too short (at most, only 2.2 Å).

In the unit cell there are still four oxygen atoms, which have to be located. They are not seen in any of the electron density maps, and to find their positions we must consider the geometry of the holes in the structure. The distance between two oxygen atoms was assumed to be ≥ 2.60 Å.

The result is shown in Fig. 5, where the forbidden areas around the O_{1a} - O_{10} atoms have been indicated for the planes $z = 0$ and $y = 0.375$. We see there that the only space free for oxygen atoms is situated within the limits $0.26 \leq x \leq 0.38$, $0.28 \leq y \leq 0.47$; $-0.25 \leq z \leq 0.25$. The symmetry of the holes is given by the symmetry of the O_{1a} - O_{10} atoms, i.e. $Pnn2$ (or $Pnnm$).

Now, two of those four areas are fully occupied by four chromate oxygen atoms

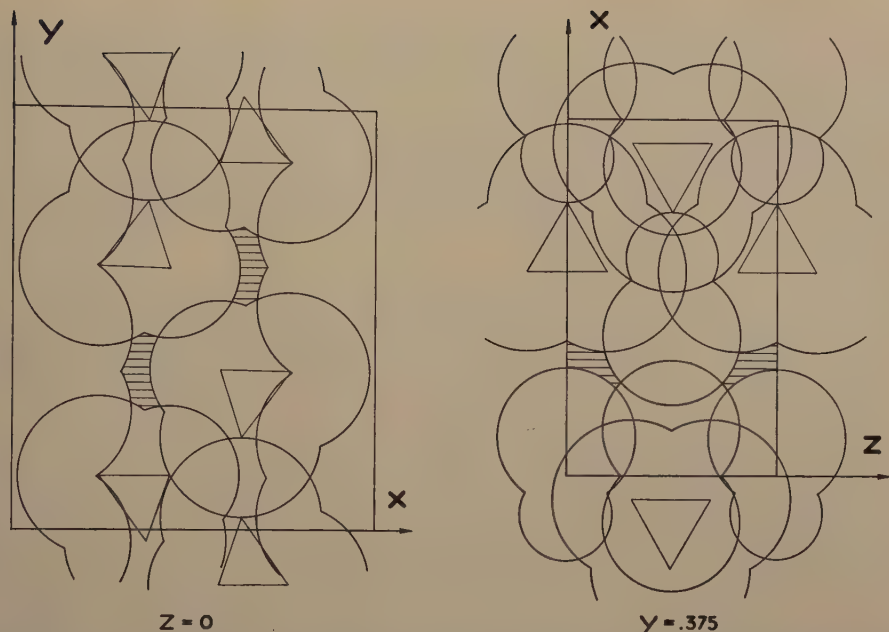


Fig. 5. Survey over the space free (shaded areas) in the planes $z=0$ and $y=0.375$ for the oxygen atoms O_{11a} and O_{11b} . The circles enclose domains around the $O_{1a}-O_{10}$ atoms forbidden for the centres of O_{11a} and O_{11b} because of the condition $O-O \geq 2.60$ Å. The triangles indicate the positions of the chromate groups Cr_1 and Cr_2 .

belonging to Cr_3 . This means that the four oxygen atoms, the positions of which we are now discussing, should be situated in the remaining two holes in the structure. The only possible arrangement is that they are situated so that they and the four chromate oxygen atoms belonging to Cr_3 together form two fourfold positions in $Pnn2$ (or one eightfold in $Pnnm$). This may not be exactly true, but, with the intensity material obtained, we have no possibility of separating the parameters of the two types of oxygen atoms.

We thus conclude that the last oxygen atoms in the unit cell should be arranged approximately as follows:

$$\begin{array}{ll} 4O_{11a} \text{ in } Pnn2:4(c) & \text{with } x \approx 0.30, y \approx 0.37_5, z \approx 0.19 \\ 4O_{11b} \text{ ,, ,, ,, } & \text{,, } x \approx 0.30, y \approx 0.37_5, z \approx 0.81 \end{array}$$

As pointed out above, the O_{11a} and O_{11b} positions are seen as a weak, rather broad maximum in $\rho(xyp)$ (Fig. 4). The Zr-O vectors corresponding to O_{11a} and O_{11b} are in agreement with the Patterson functions.

On the symmetry of the Cr_3 arrangement

We have in this way located all the zirconium, chromium, and oxygen atoms in the unit cell. The symmetry of most of the structure is given by space group $Pnn2$ (or $Pnnm$), the only exception being the Cr_3 atoms, which could be arranged according to $Pn2_1m$, $P2_1nm$, $Pnn2$ or $Pnnm$ (in the two latter cases statistically in some way or other).

If the Cr_3 atoms belong to $P2_1nm$ or $Pn2_1m$, for every fourth zirconium atom in regular fashion, one chromate group Cr_3 should be coordinated to each of the two

zirconium-oxygen chains that run through the unit cell. The chains should then be packed together either as given by $P2_1nm$ or $Pn2_1m$. In the former case the Cr_3 atoms should cause reflections $0kl$ with $k+l=\text{odd}$ to appear, whereas in the latter reflections $h0l$ with $h+l=\text{odd}$ should appear.

The photographs $h0l$ and $0kl$ were scrutinized to see if any reflections with $h+l=\text{odd}$ or $k+l=\text{odd}$ could be detected. We looked especially for the reflections $10l$, $40l$, or $60l$ and $02l$ or $06l$, which all should have high structure factors Cr_3 . Even these should, however, be just perceptible, since some other reflections of about the same magnitude could not be detected.

Though a very careful examination was made, no signs of any reflections $h0l$ with $h+l=\text{odd}$ or $0kl$ with $k+l=\text{odd}$ could be seen. The photographs thus seem to indicate that the Cr_3 atoms are not arranged according to $P2_1nm$ or $Pn2_1m$. This evidence, however, cannot be considered as quite conclusive because of the weakness of the reflections which we were seeking.

If the Cr_3 atoms are arranged according to one of the space groups $Pnn2$ or $Pnnm$, they should be situated in one of the following two ways:

1. The chromate groups Cr_3 are arranged statistically along the chains, so that there is one Cr_3 atom sometimes for neighbouring zirconium atoms, sometimes for every third, fourth, fifth, seventh etc zirconium atom.

2. The chromate groups Cr_3 are arranged as given by the space groups $P2_1nm$ or $Pn2_1m$, i.e. along each chain there are always four zirconium atoms between two chromate groups Cr_3 . The chains are then packed together, some as demanded by $Pn2_1m$ and others as demanded by $P2_1nm$. The result is a statistical arrangement according to $Pnn2$ (or $Pnnm$).

For neither of the two alternatives should the Cr_3 atoms give any extra reflections in the photographs. It is thus not possible to give any real reason in favour of one or other of them. The second possibility would give a more even distribution of the chromate ions along the zirconium-oxygen chains and the author is therefore inclined to consider the Cr_3 atoms arranged in that way.¹

On the arrangement of the hydrogen atoms

The hydrogen atoms in the unit cell should be situated close to the non-chromate oxygen atoms since the proton affinity is much larger for O^{2-} or OH^- than for CrO_4^{2-} . The twenty hydrogen atoms should therefore be distributed over those sixteen oxygen positions O_7-O_{11b} that do not belong to a chromate group Cr_3 . Since O_7-O_{10} are all in close contact with the strongly positive zirconium ions, but four of the O_{11a} and O_{11b} atoms are situated in areas with only oxygen neighbours, it seems very probable that twelve of the O_7-O_{10} positions consist of OH^- ions (the others being chromate oxygen atoms), whereas the four non-chromate oxygen positions of O_{11a} and O_{11b} are water molecules.

The formula of the crystals should thus be written as $Zr_4(OH)_6(CrO_4)_5(H_2O)_2$.

Refinement of the parameters

The structure arrived at has the symmetry given by space group $Pnnm$. The refinement of the atomic parameters could therefore be achieved by successive

¹ A statistical arrangement of the Cr_3 atoms would suggest that the compound should have a variable composition. The chromate analyses, however, did not show any variation, perhaps owing to the fact that the range of existence of $4ZrO_2 \cdot 5CrO_3 \cdot 5H_2O$ is quite limited. An increase in the amount of water during the preparation would lead to the formation of $ZrO_2 \cdot CrO_3 \cdot H_2O$.

calculations of the electron density functions, until the signs of most of the observed structure factors were known (only those of the very weakest reflections were still uncertain).

The x and y parameters of the atoms situated in the position 4(g) of $Pn\bar{n}m$ (i.e. those with $z = 0$) were determined from $\rho(xyp)$, $\rho_1(xy)$, $\rho_2(xy)$, and $\rho(xy0)$. To reduce the diffraction effects as much as possible, we also subtracted the zirconium atoms from the functions. (When calculated by the ordinary procedure, the temperature factor was found to be negligible. Probably it was roughly cancelled out by the absorption effect.)

In the same way the x and z parameters of the $O_{1a}-O_{2b}$ atoms were found from $\rho(xpz)$ and $P(upw)$. Their y parameters were more difficult to determine: $\rho(pyz)$ could not be used because of the very few observed reflections $0kl$, and in $\rho(xyp)$, $\rho_1(xy)$, and $\rho_2(xy)$ the zirconium atoms overlap the $O_{1a}-O_{2b}$ maxima. A full three-dimensional calculation of the electron density could not be performed, because of the rather uneven intensity material obtained from the photographs. However, all the calculations seemed to indicate y parameters very close to $(2n+1) \cdot \frac{1}{8}$ for all the $O_{1a}-O_{2b}$ atoms, and we concluded that $y_{O_{1a}} = y_{O_{2b}} = 0.62_5$ and $y_{O_{1b}} = y_{O_{2a}} = 0.12_5$.

The x and y parameters of O_{11a} and O_{11b} could be determined from $\rho(xyp)$, but their z parameters had to be calculated geometrically, since no definite maximum related to O_{11a} or O_{11b} could be detected in $\rho(xpz)$.

With the parameters obtained from the electron density calculations the Zr-O, Cr-O, and O-O distances within the structure were calculated. It was found that some of the O-O distances (O_4-O_{10} , O_7-O_9 , $O_{10}-O_{10}$) were too short (≈ 2.2 Å) to be correct and that the corresponding Cr-O distances were too long. Some of the oxygen parameters had thus not been correctly determined. This is not to be wondered at, however, since eight of the eleven oxygen positions in the unit cell are superimposed in pairs in $\rho(xyp)$ and $\rho_2(xy)$ and heavily influence each other in $\rho_1(xy)$ (and in $\rho(xy0)$) (see Fig. 4). Besides, the Weissenberg photographs obviously show some absorption effects (cf. below) which certainly affect the electron density functions.

The oxygen positions in question had thus to be shifted a little to get reasonable O-O and Cr-O distances in the structure. In calculating these shifts, we used the conditions that the atoms should still be situated within the highest parts of the oxygen maxima, that the errors in the zirconium and chromium positions should be negligible, that most of the atoms seemed to be arranged in pairs with $y_A + y_B = \frac{3}{4}$, and that the oxygen maxima O_7 , O_8 , and O_{11} should be reliable, since they are not superimposed by others.

The oxygen parameters were tested by calculating the structure factors $hk0$ both with the new set of parameters and also with that obtained directly from the electron density functions. They showed the same general agreement with the observed intensities, the reliability index R (Booth, 7) being about the same for both sets. However, when comparing separate reflections there were several cases where the new set of parameters showed a somewhat better agreement between observed and calculated structure factors but few where the reverse was true.

All the structure factors $hk0-hk2$, $h0l-h4l$, and $0kl-2kl$ were now calculated and compared with the observed ones. Part of the result is shown in Table 2, where the F_{hk0} , F_{hk1} , F_{h0l} , and F_{0kl} values have been listed. Table 3 gives details for some reflections with high oxygen contributions to the structure factors. As is seen, there is a good general agreement between the observed and calculated data, though there are some small discrepancies. Reflections with high k seem to be less affected by

Table 2. Calculated and observed structure factors from Weissenberg photographs of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$.

$h\ k\ l$	F calc	$ F $ obs	$h\ k\ l$	F calc	$ F $ obs	$h\ k\ l$	F calc	$ F $ obs
0 2 0	0	—	3 1 0	4	—	6 1 0	-1	—
0 4 0	-125	87	3 1 2	0	—	6 1 4	0	—
0 6 0	0	—	3 1 3	-4	—	7 1 0	-10	—
0 8 0	85	87	3 1 4	35	39	7 2 0	-40	28
0 1 0 0	0	—	3 1 5	2	—	7 3 0	-11	16
0 1 2 0	-57	55	3 1 6	0	—	7 4 0	0	—
0 1 4 0	0	—	4 0 0	-22	22	7 5 0	12	—
0 1 6 0	28	34	4 1 0	5	—	7 6 0	23	21
1 1 0	-11	7	4 2 0	0	—	7 7 0	12	20
1 2 0	-42	27	4 3 0	-4	—	7 8 0	0	—
1 3 0	-13	12	4 4 0	-39	28	7 9 0	-17	—
1 4 0	0	—	4 5 0	-5	—	7 1 0 0	-47	42
1 5 0	11	12	4 6 0	0	—	7 1 1 0	-4	—
1 6 0	39	37	4 7 0	6	—	7 1 2 0	0	—
1 7 0	16	17	4 8 0	36	29	7 1 3 0	1	—
1 8 0	0	—	4 9 0	7	11	7 1 4 0	30	35
1 9 0	-15	19	4 1 0 0	0	—	8 0 0	-17	21
1 1 0 0	13	14	4 1 1 0	0	—	8 1 0	-8	—
1 1 1 0	-3	—	4 1 2 0	-35	39	8 2 0	0	—
1 1 2 0	0	—	4 1 3 0	-3	—	8 3 0	4	—
1 1 3 0	2	—	4 1 4 0	0	—	8 4 0	45	32
1 1 4 0	-2	—	4 1 5 0	-1	—	8 5 0	8	—
1 1 5 0	7	—	4 1 6 0	42	46	8 6 0	0	—
1 1 6 0	0	—	5 1 0	4	—	8 7 0	-8	—
2 0 0	44	38	5 2 0	-59	53	8 8 0	-49	42
2 1 0	-16	9	5 3 0	7	—	8 9 0	-9	20
2 2 0	0	—	5 4 0	0	—	8 1 0 0	0	—
2 3 0	19	15	5 5 0	-6	15	8 1 1 0	0	13
2 4 0	-35	28	5 6 0	56	48	8 1 2 0	49	54
2 5 0	16	13	5 7 0	-9	—	9 1 0	3	—
2 6 0	0	—	5 8 0	0	—	9 2 0	-45	39
2 7 0	-23	24	5 9 0	6	14	9 3 0	-6	—
2 8 0	35	37	5 1 0 0	-83	79	9 4 0	0	—
2 9 0	-21	22	5 1 1 0	3	—	9 5 0	0	—
2 1 0 0	0	—	5 1 2 0	0	—	9 6 0	28	25
2 1 1 0	5	14	5 1 3 0	1	—	9 7 0	6	—
2 1 2 0	-32	41	5 1 4 0	61	64	9 8 0	0	—
2 1 3 0	4	—	5 1 5 0	0	—	9 9 0	0	—
2 1 4 0	0	—	6 0 0	-30	29	9 1 0 0	-5	13
2 1 5 0	3	—	6 1 0	9	—	9 1 1 0	-3	—
2 1 6 0	25	36	6 2 0	0	—	9 1 2 0	0	—
3 1 0	13	15	6 3 0	-13	14	10 0 0	-57	50
3 2 0	-89	73	6 4 0	19	17	10 1 0	0	16
3 3 0	15	—	6 5 0	-12	—	10 2 0	0	—
3 4 0	0	—	6 6 0	0	—	10 3 0	13	—
3 5 0	-14	15	6 7 0	18	22	10 4 0	59	41
3 6 0	86	78	6 8 0	-28	26	10 5 0	6	24
3 7 0	-19	—	6 9 0	15	—	10 6 0	0	—
3 8 0	0	—	6 1 0 0	0	—	10 7 0	-17	—
3 9 0	18	23	6 1 1 0	-5	12	10 8 0	-37	37
3 1 0 0	-28	32	6 1 2 0	30	30			

Table 2 (continued)

<i>h k l</i>	<i>F</i> calc	<i>F</i> obs	<i>h k l</i>	<i>F</i> calc	<i>F</i> obs	<i>h k l</i>	<i>F</i> calc	<i>F</i> obs
10 9 0	-8	23	1 12 1	-57	76	5 0 1	11	9
10 10 0	0	—	1 13 1	5	—	5 1 1	-12	—
10 11 0	8	—	1 14 1	0	—	5 2 1	0	—
10 12 0	23	26	1 15 1	2	—	5 3 1	14	14
11 1 0	1	—	1 16 1	41	49	5 4 1	-30	28
11 2 0	29	19	2 1 1	7	—	5 5 1	13	9
11 3 0	16	19	2 2 1	5	8	5 6 1	0	—
11 4 0	0	—	2 3 1	-10	5	5 7 1	-20	20
11 5 0	-7	—	2 4 1	0	—	5 8 1	6	14
11 6 0	-20	23	2 5 1	-7	10	5 9 1	-17	17
11 7 0	-20	24	2 6 1	31	28	5 10 1	0	—
11 8 0	0	—	2 7 1	9	9	5 11 1	5	—
11 9 0	10	—	2 8 1	0	—	5 12 1	5	—
11 10 0	26	22	2 9 1	4	—	5 13 1	3	—
11 11 0	9	18	2 10 1	-33	37	5 14 1	0	—
12 0 0	-55	43	2 11 1	-4	—	5 15 1	1	—
12 1 0	0	15	2 12 1	0	—	6 1 1	4	14
12 2 0	0	—	2 13 1	-5	—	6 2 1	-76	62
12 3 0	-13	24	2 14 1	41	56	6 3 1	8	—
12 4 0	44	28	2 15 1	2	—	6 4 1	0	—
12 5 0	-3	12	2 16 1	0	—	6 5 1	-4	—
12 6 0	0	—	3 0 1	63	61	6 6 1	45	37
12 7 0	16	23	3 1 1	14	15	6 7 1	-11	—
12 8 0	-32	31	3 2 1	0	—	6 8 1	0	—
12 9 0	6	—	3 3 1	-5	—	6 9 1	7	—
13 1 0	13	16	3 4 1	-44	28	6 10 1	-27	33
13 2 0	48	35	3 5 1	-15	19	6 11 1	4	—
13 3 0	-6	—	3 6 1	0	—	6 12 1	0	—
13 4 0	0	—	3 7 1	9	—	6 13 1	-1	—
13 5 0	-4	—	3 8 1	16	25	6 14 1	14	18
13 6 0	-29	22	3 9 1	15	22	7 0 1	-68	71
13 7 0	7	—	3 10 1	0	—	7 1 1	-5	14
0 1 1	3	—	3 11 1	-1	—	7 2 1	0	—
0 3 1	30	30	2 12 1	-2	—	7 3 1	1	—
0 5 1	-5	—	3 13 1	-5	—	7 4 1	40	30
0 7 1	-31	23	3 14 1	0	—	7 5 1	7	14
0 9 1	11	—	3 15 1	-3	—	7 6 1	0	—
0 11 1	10	—	3 16 1	-3	—	7 7 1	-2	—
0 13 1	4	—	4 1 1	-16	—	7 8 1	-21	32
0 15 1	-1	—	4 2 1	-58	50	7 9 1	-6	—
0 17 1	-16	17	4 3 1	-13	15	7 10 1	0	—
1 0 1	54	45	4 4 1	0	—	7 11 1	-1	—
1 1 1	0	—	4 5 1	18	—	7 12 1	19	22
1 2 1	0	—	4 6 1	60	59	7 13 1	2	—
1 3 1	-24	18	4 7 1	20	24	7 14 1	0	—
1 4 1	-38	26	4 8 1	0	—	8 1 1	10	—
1 5 1	-2	—	4 9 1	-21	17	8 2 1	-19	26
1 6 1	0	—	4 10 1	-30	36	8 3 1	7	—
1 7 1	24	16	4 11 1	-4	—	8 4 1	0	—
1 8 1	54	61	4 12 1	0	—	8 5 1	-14	14
1 9 1	7	—	4 13 1	5	—	8 6 1	14	9
1 10 1	0	—	4 14 1	33	52	8 7 1	-11	—
1 11 1	-8	—	4 15 1	-3	—	8 8 1	0	—

G. LUNDGREN, *A zirconium hydroxide chromate*

Table 2 (continued)

<i>h k l</i>	<i>F</i> calc	<i>F</i> obs	<i>h k l</i>	<i>F</i> calc	<i>F</i> obs	<i>h k l</i>	<i>F</i> calc	<i>F</i> obs
8 9 1	16	32	12 7 1	12	—	10 0 2	-42	36
8 10 1	-40	48	12 8 1	0	—	10 0 4	-49	43
8 11 1	1	—	12 9 1	-6	—	10 0 6	-38	39
8 12 1	0	—	12 10 1	40	24	11 0 3	-18	—
8 13 1	-4	—	13 0 1	-35	27	11 0 5	-3	—
8 14 1	26	28	13 1 1	-3	—	12 0 2	-46	43
9 0 1	-29	30	13 2 1	0	—	12 0 4	-47	38
9 1 1	12	—	13 3 1	11	—	13 0 3	-29	18
9 2 1	0	—	13 4 1	22	19	0 2 2	0	—
9 3 1	-7	12	13 5 1	8	—	0 4 2	-52	54
9 4 1	46	38	13 6 1	0	—	0 6 2	0	—
9 5 1	-7	—	13 7 1	-16	—	0 8 2	39	46
9 6 1	0	—	13 8 1	-24	21	0 10 2	0	—
9 7 1	20	14	0 0 2	136	122	0 12 2	-28	45
9 8 1	-31	35	0 0 4	132	119	0 14 2	0	—
9 9 1	18	—	0 0 6	93	100	0 16 2	7	21
9 10 1	0	—	0 0 8	69	77	0 1 3	11	—
9 11 1	-1	—	1 0 3	61	64	0 3 3	15	19
9 12 1	22	32	1 0 5	25	26	0 5 3	-12	—
10 1 1	-9	—	1 0 7	38	48	0 7 3	-21	18
10 2 1	5	—	2 0 2	26	21	0 9 3	16	—
10 3 1	-3	—	2 0 4	35	36	0 11 3	5	—
10 4 1	0	—	2 0 6	19	35	0 13 3	0	—
10 5 1	10	—	2 0 8	22	26	0 15 3	2	—
10 6 1	7	—	3 0 3	52	68	0 2 4	0	—
10 7 1	6	—	3 0 5	41	43	0 4 4	-74	66
10 8 1	0	—	3 0 7	38	45	0 6 4	0	—
10 9 1	-12	—	4 0 2	-33	26	0 8 4	57	50
10 10 1	-19	23	4 0 4	-19	17	0 10 4	0	—
10 11 1	1	—	4 0 6	-13	—	0 12 4	-42	42
10 12 1	0	—	5 0 3	14	—	0 14 4	0	—
11 0 1	-7	—	5 0 5	9	—	0 1 5	-1	—
11 1 1	-5	—	5 0 7	9	—	0 3 5	21	22
11 2 1	0	—	6 0 2	-1	—	0 5 5	-3	—
11 3 1	-4	—	6 0 4	-15	—	0 7 5	-25	15
11 4 1	31	25	6 0 6	-7	—	0 9 5	7	—
11 5 1	3	—	7 0 3	-79	82	0 11 5	9	—
11 6 1	0	—	7 0 5	-44	47	0 2 6	0	—
11 7 1	4	—	7 0 7	-55	56	0 4 6	-55	63
11 8 1	-39	40	8 0 2	7	—	0 6 6	0	—
11 9 1	-3	—	8 0 4	-12	—	0 8 6	43	52
11 10 1	0	—	8 0 6	-4	—	0 10 6	0	—
12 1 1	0	—	9 0 3	-30	39	0 12 6	-32	45
12 2 1	18	—	9 0 5	-23	29	0 1 7	4	—
12 3 1	-10	—	9 0 7	-29	45	0 3 7	12	—
12 4 1	0	—				0 2 8	0	—
12 5 1	5	—				0 4 8	-42	50
12 6 1	-13	—						

Table 3. Calculated and observed structure factors from Weissenberg photographs of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$. Reflections with high oxygen contributions.

<i>h k l</i>	<i>F</i> _{Zr} calc	<i>F</i> _{Cr} calc	<i>F</i> _O calc	<i>F</i> _{tot} calc	<i>F</i> obs	<i>h k l</i>	<i>F</i> _{Zr} calc	<i>F</i> _{Cr} calc	<i>F</i> _O calc	<i>F</i> _{tot} calc	<i>F</i> obs
0 4 0	-68	-33	-24	-125	87	5 0 1	2	22	-13	11	9
0 8 0	53	-1	33	85	87	5 4 1	-2	-15	-13	-30	28
0 12 0	-43	16	-30	-57	55	8 6 1	28	0	-14	14	9
1 6 0	18	-3	24	39	37	9 0 1	-42	-3	16	-29	30
2 5 0	0	26	-10	16	13	12 10 1	18	12	10	40	24
3 2 0	-55	-24	-10	-89	73	0 0 2	68	49	19	136	122
3 6 0	45	7	34	86	78	0 0 4	53	36	43	132	119
3 14 0	30	-7	12	35	39	0 0 6	43	28	22	93	100
4 0 0	22	-33	-11	-22	22	1 0 3	57	-19	23	61	64
4 4 0	-20	17	-36	-39	28	1 0 7	37	-12	13	38	48
4 8 0	16	9	11	36	29	2 0 2	54	-13	-15	26	21
4 16 0	11	15	16	42	46	4 0 2	20	-29	-24	-33	26
5 2 0	-59	26	-26	-59	53	4 0 4	16	-24	-11	-19	17
5 10 0	-44	-21	-18	-83	79	6 0 4	-12	12	-15	-15	—
6 0 0	-15	15	-30	-30	29	7 0 3	-26	-29	-24	-79	82
6 1 0	0	-1	10	9	—	7 0 7	-20	-21	-14	-55	56
6 4 0	14	-10	15	19	17	8 0 2	-36	16	27	7	—
6 8 0	-12	-1	-15	-28	26	8 0 6	-28	12	12	-4	—
6 12 0	11	6	13	30	30	9 0 3	-40	-3	13	-30	39
7 1 0	0	0	-10	-10	—	9 0 5	-35	-3	15	-23	29
7 6 0	39	-6	-10	23	21	11 0 5	-34	20	11	-3	—
7 10 0	-34	-1	-12	-47	42	0 1 3	0	-1	12	11	—
8 4 0	36	-7	16	45	32	0 4 2	-61	-29	38	-52	54
10 4 0	42	3	14	59	41	0 8 2	50	-1	-10	39	46
11 2 0	10	4	15	29	19	0 8 4	44	-1	14	57	50
11 10 0	8	4	14	26	22	0 12 4	-38	14	-18	-42	42
1 4 1	-62	11	13	-38	26	0 12 6	-34	12	-10	-32	45
4 2 1	-58	-17	17	-58	50	0 16 2	35	-11	-17	7	21
4 10 1	-41	-2	13	-30	36						

absorption (or: they have a lower temperature factor) than those with high *h*. Observed reflections with very low $\sin^2\theta$ are also weaker than expected. Finally, some of the weakest reflections (especially those with high $\sin^2\theta$) do not quite fit the calculated data. Accordingly the reliability index *R* (Booth, 7) was found to be rather high: 0.22 for *hk*0, 0.22 for *hk*1, 0.13 for *h*0*l*, and 0.15 for 0*kl*.

All this may, however, be explained by the fact that no real single crystals could be picked out from the preparations but all the photographs had to be taken using rather deformed crystals containing some small satellites.

Final structure proposition

The following structure is proposed for $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$. (The atomic positions given above for O_{1a} – O_{2b} , O_{11a} and O_{11b} have been combined in pairs to fit in with *Pnnm*):

Space group: no. 58 *Pnnm*. Two formula units of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$ in the unit cell.

Atomic parameters: See Table 4.

Table 4. Atomic parameters of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$.

Kind of atoms	Number of atoms	Point position	Parameters		
			<i>x</i>	<i>y</i>	<i>z</i>
Zr ₁	4	4(g)	0.049	0.125	0
Zr ₂	4	4(g)	0.049	0.625	0
Cr ₁	4	4(g)	0.364	0.088	0
Cr ₂	4	4(g)	0.364	0.622	0
Cr ₃	2	4(g)	0.197	0.375	0
O ₁	8	8(h)	0.06 ₀	0.12 ₅	0.28 ₅
O ₂	8	8(h)	0.06 ₀	0.62 ₅	0.28 ₅
O ₃	4	4(g)	0.23 ₅	0.13 ₀	0
O ₄	4	4(g)	0.23 ₅	0.62 ₀	0
O ₅	4	4(g)	0.36 ₀	0.78 ₀	0
O ₆	4	4(g)	0.36 ₀	0.97 ₀	0
O ₇ (OH)	4	4(g)	0.10 ₅	0.78 ₀	0
O ₈ (OH)	4	4(g)	0.10 ₅	0.97 ₀	0
O ₉	2O + 2OH	4(g)	0.10 ₅	0.28 ₀	0
O ₁₀	2O + 2OH	4(g)	0.10 ₅	0.47 ₀	0
O ₁₁	4O + 4H ₂ O	8(h)	0.28 ₀	0.37 ₅	0.19 ₀

The O₁, O₄, and O₅ atoms are chromate oxygen atoms belonging to Cr₂. The O₂, O₃, and O₆ atoms belong to Cr₁. The chromate oxygen atoms belonging to Cr₃ are situated in half of the positions O₉, O₁₀, and O₁₁.

The accuracy of the parameters is estimated to be ± 0.003 for Zr, ± 0.005 for Cr and ± 0.01 for O. The *z* parameters of O₁, O₂, and O₁₁ might be a little less certain. All the oxygen parameters have been rounded off to the nearest 0.005 fraction of the cell edges.

Discussion of the structure

Schematic drawings of the structure are shown in Figs. 6 and 7.

The zirconium atoms in the crystals are arranged in zigzag rows, where they are linked together by hydroxide ions and chromate oxygen atoms in the ratio 3:1, so that there is a double oxygen bridge between every pair of zirconium atoms. The resulting chains have the over-all composition $(\text{Zr}_4(\text{OH})_6\text{CrO}_4^{8+})_n$. They are drawn separately in Fig. 8. The distances Zr–Zr within the chains are 3.59₅ Å, which is within the range (3.42–4.04 Å) of Zr–Zr distances in ZrO₂ (8, 9).

The chains are then joined by other chromate ions so that every one of these chromate ions links together three chains, giving a three-dimensional arrangement (see Fig. 7). The water molecules are contained in the holes in the structure. The formula of the compound might thus be written $[\text{Zr}_4(\text{OH})_6\text{CrO}_4](\text{CrO}_4)_4(\text{H}_2\text{O})_2$ in order to mark the difference between the chromate ions Cr₃, each of which belongs to only one chain, and the joining chromate ions Cr₁ and Cr₂.

The zirconium atoms are coordinated to seven oxygen atoms, a coordination number which is rather seldom found. It is known already only in some rare earth zirconium, niobium, tantalum and uranium compounds (Wells, 10; Bailar, 11; Zachariasen, 12, 13). The coordination configuration is one of the following types: (a) a distorted trigonal prism with an atom added beyond one of the rectangular prism faces, (b) a distorted octahedron with an additional atom outside one octahedral face, or (c) a pentagonal bipyramid. The zirconium compounds— $(\text{NH}_4)_3\text{ZrF}_7$ (13, 14) and ZrOS (15)—belong to the two latter coordination types.

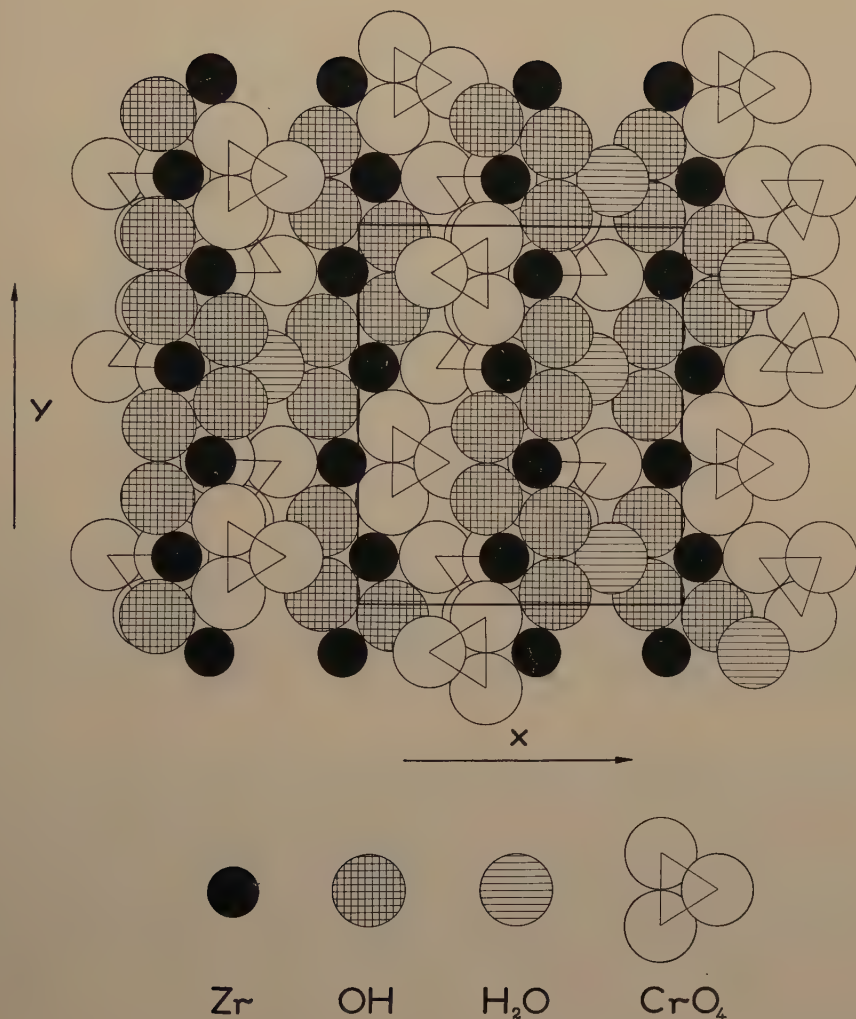


Fig. 6. Part of the structure of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$, viewed along $[001]$. The $[\text{Zr}_4(\text{OH})_6\text{CrO}_4^{8+}]_n$ chains (from left to right: with $z = \frac{1}{2}, 0, \frac{1}{2}$ and 0), most of the chromate groups and the water molecules are all shown. To make the figure clearer some chromate groups Cr_1 and Cr_2 have been omitted. The pentagons around the zirconium atoms with $z = 0$ are seen. Some possibilities for the statistical arrangement of the chromate groups Cr_3 , the water molecules, and the hydroxide ions O_9 and O_{10} are shown in the different positions of the chains with $z = \frac{1}{2}$.

The structure of $(\text{NH}_4)_3\text{ZrF}_7$ was first investigated by Hassel and Mark (16), who found that the structure contained ZrF_6^{2-} and F^- ions. Some F-F distances were, however, too short to be quite true, and this led Hampson and Pauling (14) to reinvestigate the structure. They found, mainly from arguments on interatomic distances, that the crystals should contain ZrF_7^{3-} ions with a coordination of type (b) above. This coordination around zirconium was later found also in ZrOS (15).

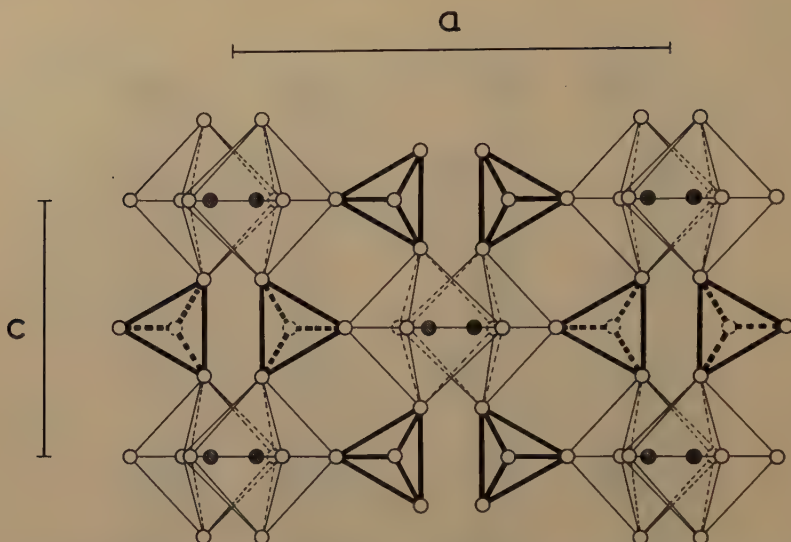


Fig. 7. A schematic drawing of part of the structure, viewed along [010], showing the way in which the chromate ions Cr_1 and Cr_2 join the $[\text{Zr}_4(\text{OH})_6\text{CrO}_4^{8+}]_n$ chains in a unit cell. Only the zirconium (shaded circles) and the O_1 – O_{10} atoms (open circles) have been indicated. To make the picture of the pentagonal bipyramids around the zirconium atoms clearer, the positions of the O_7 , O_8 , O_9 , and O_{10} atoms have been separated a little. (They should in this projection overlap completely.)

However, in 1954 Zachariasen investigated the crystal structures of $\text{K}_3\text{UO}_2\text{F}_5$ (12) and K_3UF_7 (13) on the basis of very accurately determined intensities. He found that the uranium atoms are in both compounds seven-coordinated with a coordination of type (c) above and that the positions of the potassium and uranium atoms are the same as those of the ammonium and zirconium ions in $(\text{NH}_4)_3\text{ZrF}_7$. This led him to the conclusion that the configuration of ZrF_7^{3-} proposed by Hampson and Pauling (14) is incorrect and that the fluorine atoms in ZrF_7^{3-} should also be arranged in the

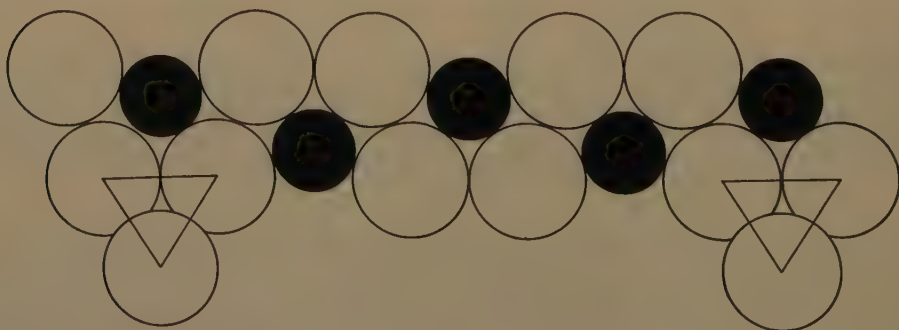


Fig. 8. The $[\text{Zr}_4(\text{OH})_6\text{CrO}_4^{8+}]_n$ chain, viewed along [001]. The atoms have been marked as in Fig. 6 with the exception that the OH^- ions have not been shaded.

Table 5. Atomic distances within the coordination polyhedra around zirconium.

For Zr_1 (Å)	For Zr_2 (Å)
Zr_1-O_1 , $O_1-Zr_1 = 2 \times 1.9_7$	Zr_2-O_2 , $O_2-Zr_2 = 2 \times 1.9_7$
Zr_1-O_3 , $O_3-Zr_1 = 2.1_7$	Zr_2-O_4 , $O_4-Zr_2 = 2.1_7$
Zr_1-O_7 , $O_7-Zr_1 = 2.2_1$	Zr_2-O_7 , $O_7-Zr_2 = 2.2_1$
Zr_1-O_9 , $O_9-Zr_1 = 2.2_1$	Zr_2-O_8 , $O_8-Zr_2 = 2.2_1, 2.2_1$
Zr_1-2O_{10} , $O_{10}-2Zr_1 = 2.2_1, 2.2_1$	Zr_2-O_9 , $O_9-Zr_2 = 2.2_1$
O_1-O_3 , $O_3-2O_1 = 2.8_3$	O_2-O_4 , $O_4-2O_2 = 2.8_3$
O_1-O_7 , $O_7-2O_1 = 2.9_3$	O_2-O_7 , $O_7-2O_2 = 3.0_4$
O_1-O_9 , $O_9-2O_1 = 3.0_4$	O_2-2O_8 , $O_8-4O_2 = 2.9_3, 3.0_4$
O_1-2O_{10} , $O_{10}-4O_1 = 2.9_3, 3.0_4$	O_3-O_9 , $O_9-2O_2 = 2.9_3$
O_3-O_7 , $O_7-O_3 = 2.5_5$	O_4-O_8 , $O_8-O_4 = 2.5_5$
O_3-O_{10} , $O_{10}-O_3 = 2.6_6$	O_4-O_9 , $O_9-O_4 = 2.6_6$
O_7-O_9 , $O_9-O_7 = 2.5_8$	O_7-O_8 , $O_8-O_7 = 2.5_8$
O_9-O_{10} , $O_{10}-O_9 = 2.5_9$	O_7-O_9 , $O_9-O_7 = 2.5_8$
$O_{10}-O_{10} = 2.5_8$	$O_8-O_8 = 2.5_8$

shape of a pentagonal bipyramid. In this way the compounds $K_3UO_2F_5$, K_3UF_7 , and $(NH_4)_3ZrF_7$ should be isostructural.

In the crystals of $Zr_4(OH)_6(CrO_4)_5(H_2O)_2$ the oxygen atoms coordinated to zirconium are arranged in the shape of an almost regular pentagonal bipyramid (see Fig. 7) which is quite in accordance with Zachariasen's considerations on the shape of the ZrF_7^{3-} ion. The Zr-O and O-O distances within the coordination polyhedra are presented in Table 5.

Considering the errors in the oxygen positions these distances are all within the normal range. [In ZrO_2 (cubic) (8), $ZrOS$ (15), $ZrOCl_2(H_2O)_8$ (17) and $ZrOBr_2(H_2O)_8$ (17) the Zr-O distances are within the range 2.09–2.37 Å; in the proposed structure of baddeleyite (9), ZrO_2 , they are 1.95–2.65 Å.]

As is seen, the distances Zr_1-O_1 and Zr_2-O_2 are a little shorter than the other Zr-O distances within the coordination polyhedra. The difference cannot, however, be considered to be significant, since the maxima in $P(upw)$, $\rho(xpz)$, and $\rho(xpz)-\rho_{Zr}(xpz)$, which determine the z parameters of O_1 and O_2 , are rather low and consequently sensitive to diffraction effects. On the other hand, it is not quite impossible that the zirconium ions influence the bonds within the joining chromate groups Cr_1 and Cr_2 distorting the chromate tetrahedra a little. The zirconium ions should thus attract the O_1 and O_2 atoms causing O_1-O_1 and O_2-O_2 within the chromate tetrahedra to become a little longer than is usual. The Cr-O bond length is not much changed by this small displacement.

The arrangement of zirconium and hydroxide ions in the crystals of $Zr_4(OH)_6 \cdot (CrO_4)_5(H_2O)_2$ is quite different from that in $ZrOCl_2(H_2O)_8$ and $ZrOBr_2(H_2O)_8$ (17). In these compounds, the zirconium atoms form finite complexes, which consist of four zirconium atoms arranged in a slightly distorted square and joined by double bridges of hydroxide ions. Four water molecules are also bound to each zirconium atom so that a coordination Zr-8O in the shape of a square Archimedean antiprism is obtained. The formula for these complexes is therefore $Zr_4(OH)_8(H_2O)_{16}^{8+}$.

The only relationship between the structures of $Zr_4(OH)_6(CrO_4)_5(H_2O)_2$, $ZrOCl_2(H_2O)_8$, and $ZrOBr_2(H_2O)_8$ thus seems to be that all three crystals have double hydroxide bridges between the zirconium atoms.

G. LUNDGREN, *A zirconium hydroxide chromate*

Preliminary investigations on the other zirconium chromate ($\text{ZrO}_2 \cdot \text{CrO}_3 \cdot \text{H}_2\text{O}$) prepared in this series show that its structure, which is tetragonal ($a = 6.868 \text{ \AA}$, $c = 29.01 \text{ \AA}$), is very closely related to that of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$. The zirconium ions seem there to be arranged in a tetragonal, two-dimensional network, built up of $\text{Zr}-\text{OH}-\text{CrO}_4$ chains joined at every second zirconium atom by a $\text{Zr}-\text{OH}$ link. A detailed description of the determination of its structure will be published later.

The distances $\text{Cr}-\text{O}$ and $\text{O}-\text{O}$ within the chromate groups in the structure of $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$ are:

For $\text{Cr}_1 \text{ (}\text{\AA}\text{)}$	For $\text{Cr}_2 \text{ (}\text{\AA}\text{)}$	For $\text{Cr}_3 \text{ (}\text{\AA}\text{)}$
$\text{Cr}_1-\text{O}_2, \text{O}_2-\text{Cr}_1 = 2 \times 1.7_9$	$\text{Cr}_2-\text{O}_1, \text{O}_1-\text{Cr}_2 = 2 \times 1.7_9$	$\text{Cr}_3-\text{O}_7, \text{O}_7-\text{Cr}_3 = 1.6_8$
$\text{Cr}_1-\text{O}_3, \text{O}_3-\text{Cr}_1 = 1.6_1$	$\text{Cr}_2-\text{O}_4, \text{O}_4-\text{Cr}_2 = 1.6_1$	$\text{Cr}_3-\text{O}_8, \text{O}_8-\text{Cr}_3 = 1.6_8$
$\text{Cr}_1-\text{O}_6, \text{O}_6-\text{Cr}_1 = 1.6_1$	$\text{Cr}_2-\text{O}_5, \text{O}_5-\text{Cr}_2 = 1.6_1$	$\text{Cr}_3-\text{O}_{11}, \text{O}_{11}-\text{Cr}_3 = 2 \times 1.6_8$
$\text{O}_2-\text{O}_3 = 2.9_6$	$\text{O}_1-\text{O}_1 = 2.9_6$	$\text{O}_7-\text{O}_8, \text{O}_8-\text{O}_7 = 2.5_9$
$\text{O}_2-\text{O}_3, \text{O}_3-\text{O}_2 = 2.8_0$	$\text{O}_1-\text{O}_4, \text{O}_4-\text{O}_1 = 2.8_0$	$\text{O}_7-\text{O}_{11}, \text{O}_{11}-\text{O}_7 = 2 \times 2.7_4$
$\text{O}_3-\text{O}_6, \text{O}_6-\text{O}_3 = 2.7_5$	$\text{O}_1-\text{O}_5, \text{O}_5-\text{O}_1 = 2.7_5$	$\text{O}_8-\text{O}_{11}, \text{O}_{11}-\text{O}_8 = 2 \times 2.7_4$
$\text{O}_3-\text{O}_6, \text{O}_6-\text{O}_3 = 2.6_2$	$\text{O}_4-\text{O}_5, \text{O}_5-\text{O}_4 = 2.6_2$	$\text{O}_{11}-\text{O}_{11} = 2.6_1$

These distances are all within the normal range, though O_1-O_1 and O_2-O_2 have been elongated a little due to their nearness to the zirconium atoms (see above).

The remaining $\text{O}-\text{O}$ contacts in the structure are:

$\text{O}_1-\text{O}_4, \text{O}_4-\text{O}_1 = 2.8_1 \text{ \AA}$	$\text{O}_5-\text{O}_{11}, \text{O}_{11}-\text{O}_5 = 2 \times 2.9_8 \text{ \AA}$
$\text{O}_1-\text{O}_5, \text{O}_5-\text{O}_1 = 2.7_4 \text{ \AA}$	$\text{O}_6-\text{O}_{10}, \text{O}_{10}-\text{O}_6 = 2.9_7 \text{ \AA}$
$\text{O}_3-\text{O}_3, \text{O}_3-\text{O}_3 = 2.8_1 \text{ \AA}$	$\text{O}_6-\text{O}_{11}, \text{O}_{11}-\text{O}_6 = 2 \times 2.9_8 \text{ \AA}$
$\text{O}_3-\text{O}_6, \text{O}_6-\text{O}_3 = 2.7_4 \text{ \AA}$	$\text{O}_8-\text{O}_{11}, \text{O}_{11}-\text{O}_8 = 2 \times 2.8_4 \text{ \AA}$
$\text{O}_5-\text{O}_6, \text{O}_6-\text{O}_5 = 2.5_9 \text{ \AA}$	$\text{O}_{10}-\text{O}_{11}, \text{O}_{11}-\text{O}_{10} = 2 \times 2.8_4 \text{ \AA}$
$\text{O}_5-\text{O}_9, \text{O}_9-\text{O}_5 = 2.9_7 \text{ \AA}$	

All these distances are within the normal range. The structure is thus well stabilized by $\text{O}-\text{O}$ contacts, every oxygen atom being in contact with 9–11 neighbouring atoms.

SUMMARY

A crystalline, basic zirconium chromate with the mole ratio $\text{ZrO}_2:\text{CrO}_3:\text{H}_2\text{O} = 4:5:5$ has been prepared by hydrothermal methods. On the basis of a structure determination it has been given the formula $\text{Zr}_4(\text{OH})_6(\text{CrO}_4)_5(\text{H}_2\text{O})_2$.

The crystals are orthorhombic with $a = 11.63 \pm 0.01 \text{ \AA}$, $b = 13.65 \pm 0.01 \text{ \AA}$, and $c = 6.878 \pm 0.005 \text{ \AA}$. The unit cell contains two formula units.

A structure is proposed with atomic positions according to space group no. 58 $Pnmm$. The parameters have been determined mainly by Patterson and Fourier methods.

The structure is built up of infinite chains with the over-all composition $[\text{Zr}_4(\text{OH})_6\text{CrO}_4^{8+}]_n$. The chains are then connected by other chromate groups to a three-dimensional arrangement. The water molecules are contained in holes in the structure.

The zirconium atoms are coordinated to seven oxygen atoms in the shape of an almost regular pentagonal bipyramid.

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Resolution and configuration of 2-thienylglycolic acid

A new determination of the configuration of mandelic acid

By SALO GRONOWITZ

With 7 figures in the text

In connection with work on optically active thiophene compounds the present author has resolved 2-thienylglycolic acid (1) into its antipodes. The dextrorotatory enantiomorph was obtained through fractional crystallization of the (-)-phenethylamine salt, and the levorotatory form through fractional crystallization of the cinchonidine salt. As in both cases the same maximum activity was obtained, it is justifiable to assume that the preparations having a m.p. $83-84^{\circ}$ represent the optically pure antipodes. Fujise (2) has earlier obtained the dextrorotatory form through fermentation of 2-thienylglyoxal. Like the inactive modification, the optically active forms darken in light and decompose on heating at 100° . However, when stored in refrigerator in the dark they are rather stable. After one year, less than 5 % of the activity was lost.

In order to determine the absolute configuration of the active 2-thienylglycolic acid, the Fredga method of quasi-racemate (3) was applied so as to connect this acid sterically to mandelic acid. The thermal method for detecting quasi-racemates seemed not feasible, as 2-thienylglycolic acid is unstable at its melting-point. It was, however, possible to obtain the phase-diagram for 2-thienylglycolic acid through instantaneous melting-point determinations (Fig. 1).

This is of some interest, as information on the racemate-forming tendency, as expressed by the difference between the melting-points of the racemic compound and the eutectics, or, as suggested by Pettersson (4), as the ratio of this difference to the difference between the melting-points of the antipodes and the eutectics, can thus be obtained. From Fig. 1 it can be seen that the melting-points of the antipode,

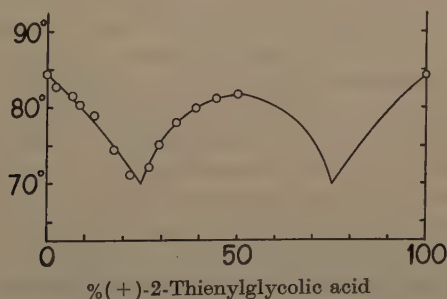


Fig. 1. (+)- and (-)-2-Thienylglycolic acid.



Fig. 2. (a) *rac.*-Mandelic acid (b) *rac.*-2-Thienylglycolic acid.

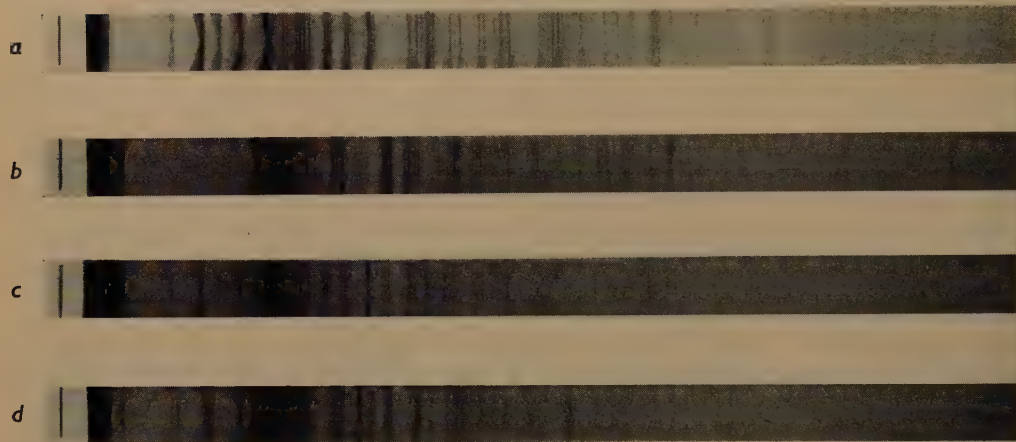


Fig. 3. (a) $(-)$ -2-Thienylglycolic acid. (b) $(-)$ -Mandelic acid. (c) Equimolar amounts of $(-)$ -mandelic acid and $(-)$ -2-thienylglycolic acid. (d) Equimolar amounts of $(+)$ -mandelic acid and $(-)$ -2-thienylglycolic acid.

racemate and eutectic are 84° , 81° and 70° respectively. Angus and Owen (5) have studied the melting-point diagram for mandelic acid and found the corresponding melting-points to be 133° , 118° and 116° .

The racemate-forming tendency of 2-thienylglycolic acid seems thus to be greater than that of mandelic acid. There is thus no reason to assume that the 2-thienyl group has a lower tendency for racemate formation than the phenyl group (6), since this is the second case (7) where the contrary has been found.

2-Thienylglycolic acid was related to mandelic acid by performing the phase analysis by X-ray powder photographs and infrared spectroscopy (8).

Powder photographs confirm that inactive 2-thienylglycolic acid as well as inactive mandelic acid are true racemates (Fig. 2). They also show that these acids as well as the active acids are not isomorphous. The powder photograph of an equimolar mixture of $(-)$ -2-thienylglycolic acid and $(-)$ -mandelic acid is additively composed of the lines of the components. An equimolar mixture of $(-)$ -2-thienylglycolic acid and $(+)$ -mandelic acid gives a different powder photograph, however, thus showing it is a quasi-racemate (Fig. 3).

The IR-spectrum of active 2-thienylglycolic acid is different from that of the inactive acid (1) which gives additional evidence concerning the true racemate nature of this acid. The equimolar mixture of $(-)$ -2-thienylglycolic acid and $(-)$ -mandelic

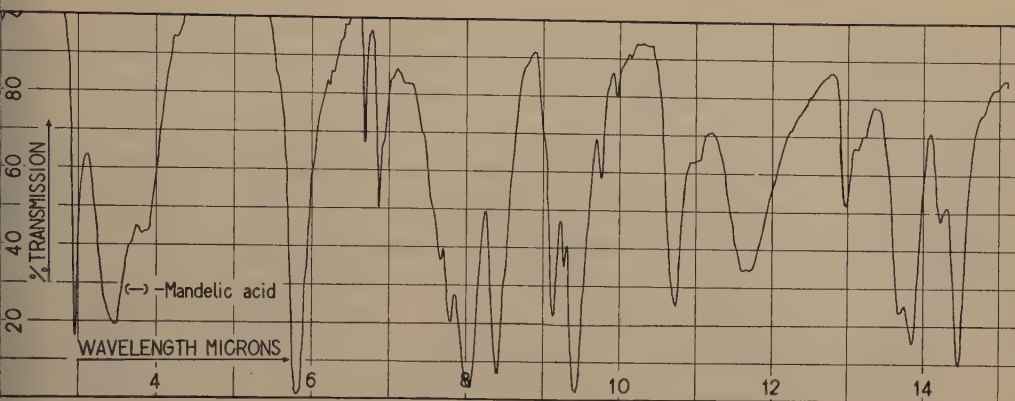


Fig. 4. IR-spectrum of (-)-mandelic acid.

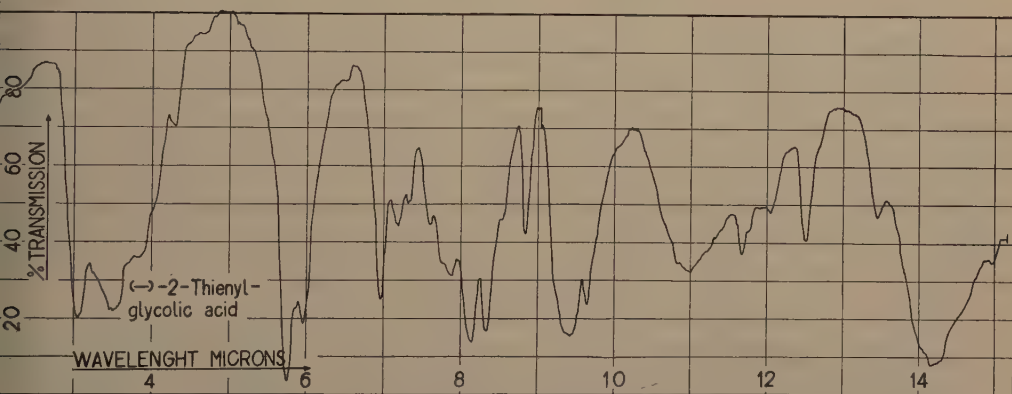


Fig. 5. IR-spectrum of (-)-2-thienylglycolic acid.

acid has a spectrum which, apart from some small differences, is additively composed of the spectra of the components. The spectrum of the equimolar mixture of (+)-mandelic acid and (-)-thienylglycolic acid, however, shows appreciable differences. (-)-2-Thienylglycolic acid has the maxima in the carbonyl stretching region at $5.75\ \mu$ and $5.98\ \mu$. In the racemic form only one top at $5.87\ \mu$ appears. Both these tops are present in the (-)-mandelic acid, (-)-thienylglycolic acid mixture. (In the mandelic acids this top is at $5.80\ \mu$.) In the (+)-mandelic acid (-)-thienylglycolic acid mixture, however, a single top appears at $5.87\ \mu$. Several other marked differences appear in the finger-print region as can be seen from Figs. 4-7.

The optical rotation of 2-thienylglycolic acid in different solvents also shows a marked parallelism with that of mandelic acid in the same solvents (Table 1). Only the values in ethyl acetate fall a little out of line.

The results obtained establish that 2-thienylglycolic acid and mandelic acid with the same mode of rotation also have the same configuration.

Although many studies on the configuration of mandelic acid have been carried out, none of them has been completely convincing. Winter (9) used the solubility of

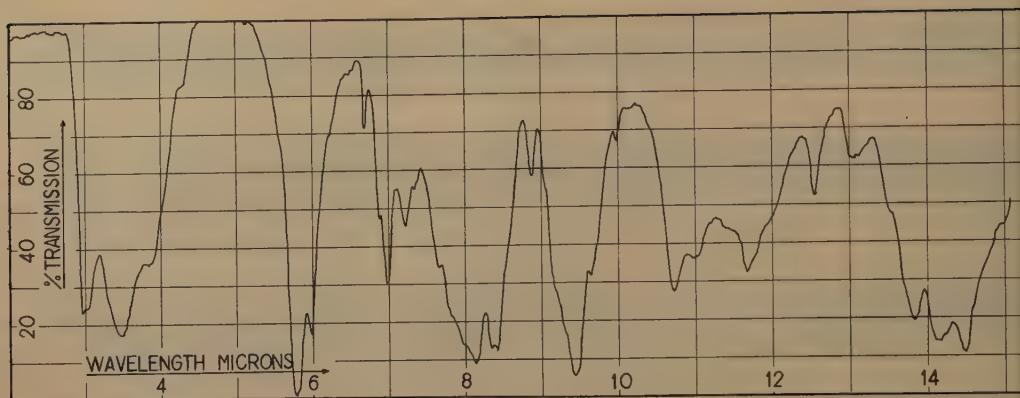


Fig. 6. IR-spectrum of equimolar amounts of (-)-mandelic acid and (-)-2-thienylglycolic acid.

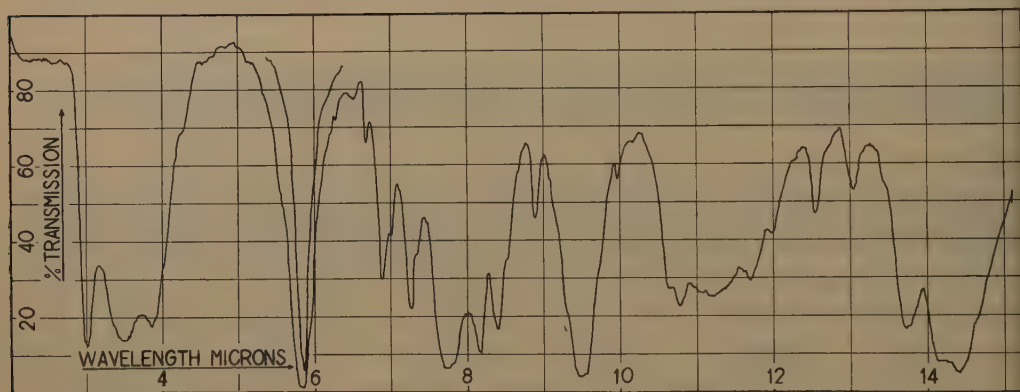


Fig. 7. IR-spectrum of equimolar amounts of (+)-mandelic acid and (-)-2-thienylglycolic acid.

alkaloid salts of α -hydroxy acids (Winter's rule). Matell (10), however, has shown that this rule does not hold even for structurally related acids. Several authors (11) have used optical comparison between derivatives of lactic acid, hexahydromandelic acid and mandelic acid (displacement rule). Such comparisons, however, must be done very carefully and there are exceptions which makes this method not completely convincing. Biochemical reactions have also been used for establishing the configuration of mandelic acid, but conflicting results were obtained (12). *Aspergillus griseus* thus for example destroys (-)-mandelic acid most rapidly as well as lactic acid with the same direction of rotation. *Aspergillus niger*, however, prefers (+)-mandelic acid, so it is apparent that not only steric factors influence the activity of microorganisms.

The need for an unequivocal determination of the configuration of mandelic acid was felt by Mislow (13), who some years ago chemically related (-)-mandelic acid to (-)-ethyl- α -methyl benzyl ether, which through the work of Levene *et al.* (14) has been chemically related to lactic acid. Mislow does not discuss the reliability of the determinations carried out by Levene (14). However, at least one of the reactions was carried out under such vigorous conditions and proceeded apparently with

Table 1. Optical activity of 2-thienylglycolic acid and mandelic acid.

Solvent	[M] _D ²⁵ of acid	
	(+)-2-Thienylglycolic acid	(+)-Mandelic acid
Acetic acid	+ 172°	+ 269°
Water	+ 158°	+ 242°
Ethanol	+ 140°	+ 228°
Acetone	+ 135°	+ 225°
Ethyl acetate	+ 135°	+ 237°
Ether	+ 112°	+ 212°
Water (ion)	+ 91°	+ 179°

such extensive loss of optical activity that at least some doubt exists about the validity of his conclusions. Through this twelve steps relation (–)-mandelic acid was assigned to the D-series of α -hydroxy acids. This correlation is also supported by Prelog's (15) work on asymmetric syntheses.

Some years ago Fredga (16) introduced a very convenient method for the determination of configuration of phenylsubstituted optically active compounds. They were related through quasi-racemate to the corresponding 2-substituted thiophenes, which through Raney-nickel desulphurization were connected with aliphatic compounds. This method has been used by Fredga and coworkers for the determination of several optically active phenyl compounds with carbon-substituent at the asymmetric center.

It has now been found that the Fredga thiophene method can also be used for a compound having a hydroxyl group at the asymmetric center if special precautions are observed during the desulphurization. The products formed by the action of Raney-nickel depend to a high degree on the type of solvent used, the method of preparing the catalyst, reaction time and temperature. Bonner (17) thus has found that ethyl mandelate is almost completely dehydroxylated in ethanol and found with optically active ethyl atrolactate that this dehydroxylation proceeded with retention of configuration.

It seemed therefore suitable to desulphurize in alcohol free medium, especially since in one trial experiments conducted in the usual manner (16), indications of the formation of caproic acid were obtained. α -Hydroxycaproic acid has earlier been obtained by the present author (1) through desulphurization with Raney-nickel alloy in strong alkali. This way proved to be impracticable, however, as preliminary racemization experiments with 2-thienylglycolic acid show that the acid racemized at 80° although it seemed to be stable in 0.1 N sodium hydroxide at room temperature. After six hours at this temperature, 35 % of the activity was lost.

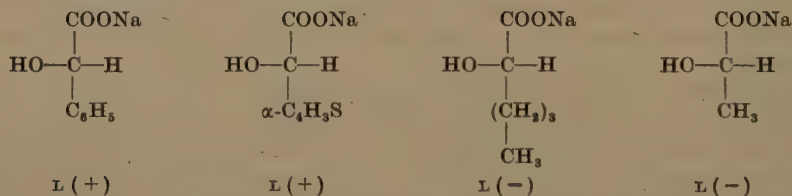
Experiments therefore were undertaken with a W-2 catalyst, prepared according to Mzingo (18) except that the last washings with 95 % alcohol and absolute alcohol were omitted. The neutral catalyst was used at once for the desulphurization of 2-thienylglycolic acid neutralized with sodium bicarbonate in water. In this way good yields of α -hydroxycaproic acid were obtained after two hours' reflux. The completeness of desulphurization is easily checked through UV-spectroscopy. 2-Thienylglycolic acid has a strong absorption band around 235 m μ with $\epsilon = 7800$, while

the extinction for the aliphatic α -hydroxycaproic acid is only about 10 at this wavelength.

When the desulphurization was performed in the same manner with the active acid, however, complete racemization occurred. This was rather unexpected. It is possible that the nickel is in some way involved in the racemization. Bonner (17) has found that optically active derivatives of 2-phenylpropionic acid are slowly racemized by Raney-nickel in refluxing ethanol.

When the desulphurization of (+)-thienylglycolic acid was carried out at room temperature, however, optically active α -hydroxycaproic acid was obtained. The sodium salt of this acid was levorotatory, $[\alpha]_D^{25} = -14.0^\circ$. Levene *et al.* (19) give $[\alpha]_D^{20} = +14.8^\circ$ for the sodium salt obtained through fractional crystallization of the cinchonidine salt, and Horn and Pretorius (20) give $[\alpha]_D^{18} = -14.1^\circ$ for the salt obtained from (-)-malic acid. Levene *et al.*, however, measured the rotation at about 40 times higher concentration. This indicates that the desulphurization has proceeded without any appreciable racemization. The configuration of (-)-sodium α -hydroxycaproate relative to lactic acid has been determined by Levene *et al.* (21) in a complex series of steps by chemical methods, and recently in a more direct and convincing manner relative to malic acid by Horn and Pretorius (20) through anodic coupling of the half ester of (-)-malic acid and butanoic acid. In both cases α -hydroxycaproic acid is connected to acids, whose absolute configurations are known.

The following relations are thus established. (The rotation values are given for salts in water to avoid confusion, as α -hydroxycaproic acid is levo- or dextrorotatory depending upon solvent and concentration.)



In this connection it should be mentioned that Timmermans *et al.* (22) tried to relate mandelic acid to lactamide through quasi-racemate. However, as Fredga (3) has pointed out, the components are so unlike chemically, that it must be doubted whether the molecular compound is really comparable with a racemic compound. As in the case of dichlorosuccinic acid and lactamide, the molecular compound could be salt-like in nature and hence hardly permits any conclusions regarding the relative configurations of the components. The change of a phenyl group for a 2-thienyl group, however, is a very small one. The phenyl and the analogues thienyl compounds are often, but not always isomorphous, and in some cases evidence for the crystallographical similarity between the quasi-racemate and the racemate has been obtained (23).

Experimental

Preliminary tests on resolution were made with 0.005 mole of optically active acid and 0.005 moles of base in dilute alcohol. Most alkaloids, namely quinine, quinidine, cinchonine, brucine and strychnine gave soluble salts. Gummy precipitates were obtained with yohimbine and morphine. A crystalline salt formed with (+)-benzdrine but the acid recovered was inactive.

With (-)-phenethylamine in ethyl acetate, however, an acid with $[\alpha]_D^{25} = +21.8^\circ$, and with cinchonidine an acid $[\alpha]_D^{25} = -56.5^\circ$ were obtained in 20 % ethanol. These bases were therefore used for the resolution.

Optical resolution of 2-thienylglycolic acid

29.7 g (0.188 mole) of 2-thienylglycolic acid and 55.4 g (0.188 mole) of cinchonidine were dissolved by heating in 1400 ml of water and 300 ml of alcohol and the solution left in a refrigerator for 24 hours. The precipitate was filtered off, washed with cold water, and dried. About 0.4 g of the salt was set apart, the acid isolated and the optical activity measured in water. The remaining salt was recrystallized from dilute ethanol and the process repeated until the optical activity of the acid remained constant. The course of the resolution is seen below.

Crystallization	1	2	3	4	5
g salt	42	33	28	23.5	19.5
ml water	1400	1080	930	800	700
ml ethanol	300	230	180	150	125
$[\alpha]_D^{25}$ of acid	-73°	-96°	-95°	-95°	-96°

The mother liquor from the first crystallization with cinchonidine was evaporated in vacuum under nitrogen, and 43 g of salt was obtained. This salt yielded 13 g (0.082 mole) of acid having $[\alpha]_D^{25} = +72^\circ$. This acid was dissolved in 680 ml of hot ethyl acetate, and 9.9 g (0.082 mole) of (-)- α -phenylethylamine was added. The salt deposited almost instantly and was filtered off when the solution had cooled. The progress of the resolution is seen below.

Crystallization	1	2	3	4
g salt	19.5	15.5	13.2	9.2
ml ethylacetate	680	600	550	600
ml methanol	—	50	40	50
$[\alpha]_D^{25}$ of acid	—	$+94^\circ$	$+96^\circ$	$+97^\circ$

(-)-2-Thienylglycolic acid

The cinchonidine salt (19 g) was decomposed with 1 N sulphuric acid and the active acid extracted 20 times with ether. The ether was removed in vacuo under nitrogen and the acid recrystallized from benzene. The yield of pure acid was 4.2 g and the m.p. $83-84^\circ$.

0.07107 g acid: 4.47 ml 0.1002 N NaOH.

$C_8H_6O_3S$ (158.2). Equiv. wt. calc. 158.2, found 158.7.

0.2037 g acid made up to 20.00 ml with water:

$2\alpha_D^{25} = -2.003$, $[\alpha]_D^{25} = -98.3^\circ$, $[M]_D^{25} = -155.5^\circ$.

(+)-2-Thienylglycolic acid

8.0 g of the phenylethylamine salt was decomposed with 1 N sulphuric acid and worked up in the same manner as above. After one recrystallization from benzene, 2.8 g of pure acid m.p. $83-84^\circ$ was obtained.

0.11326 g acid: 7.11 ml 0.1002 N NaOH

$C_8H_6O_3S$ (158.2). Equiv. wt. calc. 158.2, found 159.0.

Weighed amounts of acid were made up to 20 ml with different solvents and the optical activity measured (Table 2).

Table 2.

Solvent	mg of acid	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Acetic acid	100.67	+ 1.094°	+ 108.7°	+ 172.0°
Water	202.02	+ 2.012°	+ 99.6°	+ 157.6°
Ethanol	111.26	+ 0.980°	+ 88.1°	+ 139.4°
Acetone	106.50	+ 0.908°	+ 85.3°	+ 134.9°
Ethyl acetate	167.60	+ 1.427°	+ 85.1°	+ 134.6°
Ether	111.67	+ 0.792°	+ 70.9°	+ 112.2°
Water (ion)	141.73	+ 0.823°	+ 51.0°	+ 91.9°

The optical rotation of 0.1246 g of (+)-2-thienylglycolic acid in 20 ml of water was measured at different wavelengths. The results are given in Table 3.

Table 3.

Wavelength Å	$2\alpha_D^{25}$	$[\alpha]_D^{25}$
6438	+ 0.981°	+ 78.7°
5893	+ 1.213°	+ 97.3°
5461	+ 1.429°	+ 114.6°
5086	+ 1.690°	+ 135.6°

Raney-nickel desulphurization

Raney-nickel catalyst was prepared as described by Mozingo (18) from 50.5 g of sodium hydroxide in 200 ml of water and 40 g Raney-nickel alloy, except that the washings with alcohol were omitted. The catalyst was added to a solution of 1.0 g (0.00632 mole) of 2-thienylglycolic acid and 0.54 g (0.00642 mole) sodium bicarbonate in 200 ml of water, and the mixture was stirred at room temperature for 18 hours. The nickel was filtered off and washed with water and 0.1 *N* sodium hydroxide solution. The combined acidified water phases were continuously extracted with ether. The ether solution was dried, and the ether was distilled off *in vacuo*, leaving 0.63 g of a crystalline residue m.p. 55–58°. After one recrystallization from 20 ml of petroleum-ether (b.p. 45–50°) and 3 ml of ether 0.36 g of α -hydroxycaproic acid m.p. 58–60° was obtained. This acid gave no m.p. depression with an authentic sample of α -hydroxycaproic acid (24).

0.10140 g acid: 7.68 ml 0.1002 *N* NaOH.

$C_6H_{12}O_3$ (132.1). Equiv. wt. calc. 132.1, found 131.7.

0.02287 g made up to 10.00 ml with water and the UV-absorption measured at 235 $m\mu$ gave $D = 0.277$, $\epsilon = 16$. (2-Thienylglycolic acid gave $\epsilon = 7800$ at this wavelength.)

When 1.0 g of (+)-2-thienylglycolic acid ($[\alpha]_D^{25} = +98^\circ$ in water, $[\alpha]_D^{25} = +51^\circ$ for the sodium salt in water) was treated in exactly the same manner, 0.32 g acid m.p. 59–60° was obtained after one recrystallization from petroleum-ether (25 ml) and ether (3 ml).

Anal. 74.36 mg; 5.60 ml 0.1002 *N* NaOH; -6.685 mg; 13.366 mg CO₂ and 5.395 mg H₂O.
C₆H₁₂O₃ (132.1) calc. Equiv. wt. 132.1, C 54.55, H 9.16;

found Equiv. wt. 132.5, C 54.56, H 9.03.

0.22066 g acid were neutralized with 16.66 ml 0.1002 *N* sodium hydroxide and made up to 20.00 ml with water.

$2\alpha_D^{25} = -0.36^\circ$, $[\alpha]_D^{25} = -14.0^\circ$.

0.01794 g acid were made up to 10.00 ml and the UV-absorption measured at 235 mμ, $n_D = 0.233$, $\epsilon = 17$.

Optical activity, melting-points, X-ray powder photographs and infrared spectra were determined as described earlier (25). The UV-measurements were carried out with a Beckman model DU spectrophotometer.

SUMMARY

2-Thienylglycolic acid has been resolved into its antipodes. Its steric relationship to mandelic acid has been established by the quasi-racemate method, and through Raney-nickel desulphurization it has been related to α -hydroxycaproic acid. The assignment of (-)-mandelic acid to the *D*-series of α -hydroxy acids has thus been confirmed.

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Institute of Organic Chemistry, Royal Agricultural College, Uppsala. February 1958.

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Influence of ionizing radiation on potato tubers

By MAIRE JAARMA

With 3 figures in the text

ABSTRACT

The effect of γ -irradiation on the sugar content in tubers of three different potato varieties has been investigated. Qualitative and quantitative analyses of alcohol-soluble sugars and the sugars in press-juices of the tubers have been carried out. The potatoes were stored at $+5^{\circ}\text{C}$ and at different times after harvest treated with various dosages of γ -irradiation in the range from 400 to 2×10^6 rad. Dosages less than 4000 rad had no influence on the three major sugar components of the tubers: sucrose, glucose, and fructose. By dosages in the range 4000 to 16,000 rad a marked increase in the sucrose content and a smaller increase in the glucose content, but a decrease in the fructose content was observed. These changes were reversible and disappeared after storage for some months at $+5^{\circ}\text{C}$. The high dosages (160,000 and 200,000 rad), however, resulted in a 900 % increase in the sucrose and about a 100 % increase in the glucose content, but a decrease in the fructose content of about 75 %. At 18,000 rad and higher dosages there was a complete inhibition of sprouting. At low dosages (400 to 800 rad), however, a pronounced stimulation of sprouting was observed in some cases.

Introduction

Production of ions and excited molecules throughout an irradiated medium is only the first of a series of effects. These include biological and chemical changes, both of which are of actual interest. The sensitivities of different organisms, and of different cells of a complex organism, to these changes vary widely, but, in general, the larger and more complex a cell or organism, the more sensitive it is to radiation damage.

As reported in a previous paper (1) chronic γ -irradiation decisively changed the mono- and disaccharide composition in *Vicia faba*. However, no changes were found in the sugar metabolism of chronic γ -irradiated plants of potato, *Solanum tuberosum*, which had received total dosages as high as 20,000 rad.¹ The investigation (3) also showed that, at this radiation level, there was no inhibition of the plant growth nor of the development of the tubers. When irradiating the tubers, however, the sugar content was influenced at an acute dosage as low as 4000 rad.

Since Sparrow and Christensen in 1950 and 1954 reported that sprouting of potato tubers could be retarded (4) or inhibited (5) with X-rays and γ -rays, wide-spread

¹ The unit rad is used according to the recommendations of the *International Commission on Radiological Units* (2).

interest has been shown in investigating the effects of the irradiation of potatoes and other vegetables.

The investigation presented here was undertaken to determine the changes in sugar contents in potato tubers irradiated with different dosages of γ -rays at various times after harvest. Attempts were also made to determine the upper dosage limit, which could be used without severely damaging the tubers (even after prolonged storage) and also to determine any stimulating effects caused by low doses.

Materials and methods

Most of the results were obtained with two potato varieties: Eva, an early variety, which was potato wart immune and virusfree, and Bintje, a middle early variety. Supplementary examinations were in some cases made with a late variety, President. In 1955 the tubers used in this investigation were harvested at different dates, Eva in August, and Bintje in September. In 1956 Eva and Bintje were harvested at the same date in September, and in 1957 all the three varieties were harvested in September, the lots used in these examinations being harvested on the same day. The tubers were sorted by hand and selected so that the weight of 100 tubers was between 6 and 10 kg. The potatoes were stored at $+5^{\circ}\text{C}$. The γ -irradiation was carried out at $+20^{\circ}\text{C}$ at different distances from a ^{60}Co -source, described by Ehrenberg (6). During exposure at 20 cm distance the tubers were kept in special masonite lidded boxes (Fig. 1) each having a capacity of 1.4 kg. At longer distances from the radiation source ordinary masonite lidded boxes, measuring $29 \times 27 \times 8$ cm, and with a capacity of 3.75 kg were used. These boxes were turned round at the middle of the exposure period. Some of the irradiations were performed at $+5^{\circ}\text{C}$. In order to obtain exactly the same conditions of temperature and humidity as in the irradiated samples, the control tubers were kept for the irradiation period in a part of the irradiation room shielded by iron-concrete and lead.

For the sugar determinations the tubers were rapidly peeled by hand and cut in pieces. The sugar contents as well in the body of the tuber as in the peels were investigated. 25 g of each sample was immediately extracted in 80 % ethanol. Another sample, weighing 100 g, was simultaneously minced in a Turmix apparatus and squeezed through a Hafico press with a special inset of stainless steel¹ giving a pressure of 4000 kg/cm^2 . The press-juice was then centrifuged at 3500 rpm. Analyses of evaporated alcohol extracts were performed simultaneously. The methods for the sugar determinations were identical with those reported previously (1). The elutions of the sugars separated on paper chromatograms were, however, very time-consuming and therefore a simplified method was also used. The order of magnitude of the amounts of sugars in the solution to be tested was first determined on a preliminary paper chromatogram. As the concentrations of the different sugars in the mixture varied, suitable dilutions were made giving consideration to each particular sugar. Papers were then "loaded" with a series of different known amounts of the reference sugar and with spots of different concentrations of the test solution in order to encircle the spots of the separated sugars. The results were ascertained by determining the smallest detectable amount visible with the different sprays. The error with this method was only 2 %, i.e. not greater than the error in the elution method.

The irradiations by 180,000 rad were performed at a rate of 2200–1800 rad per hour (1956–1958). As certain after-effects could be expected, some of the tubers were analyzed one or two days after the irradiation, the rest of the lot being stored at $+5^{\circ}\text{C}$ and then reanalyzed at different intervals. Some experiments also were performed by doses of 1.3×10^6 and 2×10^6 rad. Tubers exposed

¹ For the construction thanks are due to *Lindhés Mek. Verkstad*, Stockholm.

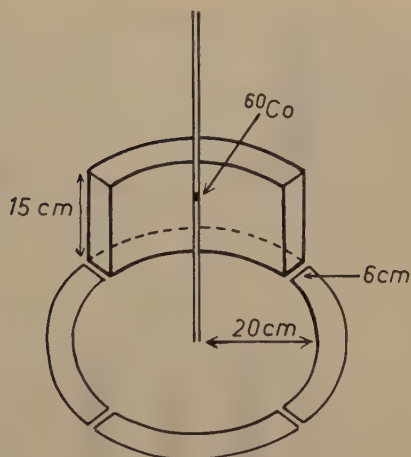


Fig. 1. Irradiation boxes used at 20 cm distance from the ^{60}Co -source.

to the latter dosage have been stored at room temperature for two years and have then been analyzed. The experiments by dosages from 400 to 16,000 rad were carried out at lower dose-rates.

The dose-rates were partly determined by measuring the oxidation of ferrous sulfate in 0.8 *N* sulfuric acid according to Ehrenberg and Sæland (7), and partly with Victoreen ionization chambers.

Results

Effects on the sugar contents

Fig. 2 illustrates the changes in the sugar contents in tubers of the Bintje variety, which were irradiated in November 1956 by 4000–16,000 rad and 160,000 rad. The tubers were placed at different distances from the source of irradiation so that the duration of irradiation was the same for all three doses. Analyses carried out 4 days after irradiation showed that the sucrose content had increased with the increasing radiation dose, from the level of the control tuber (0.2 %) to 3 % at the highest dose administered, i.e. the increase amounted to not less than 1400 %. The glucose content had increased by 300 % at 160,000 rad, but the fructose concentration at this dose had decreased by 70 %. The results of the analyses carried out on the same material five months later showed that the sugar content at the lower irradiation doses returned to the level of the normal tuber, while the changes occurring at the highest dose levels remained nearly constant. In Table 1 some of the sugar values are given for the Bintje tubers, which were irradiated, at different times after harvest, by a dose of 180,000 rad. The results for the peels are also stated. It will be noted that these are not much lower than the corresponding values found for peeled tubers. Also included in this table are the values obtained for the doses 1.3×10^6 and 2×10^6 rad.

Using qualitative paper chromatographic analyses, extremely small quantities of a reducing sugar, probably xylose, were detected in the peels of irradiated and non-irradiated tubers. This substance was not found in the peeled potatoes. The presence of maltose was confirmed in both the peels and in the body of tubers. However, this sugar was also identified only by paper chromatography, using maltose as the reference substance.

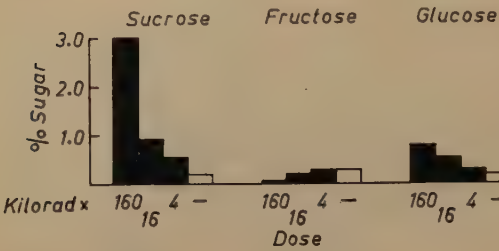


Fig. 2. Changes in the sugar contents (per cent of fresh weight) in potato tubers, Bintje variety, after irradiation with 4000–160,000 rad. ■, irradiated; □, control.

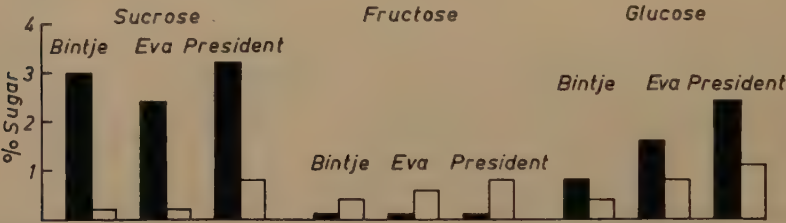


Fig. 3. Sugar contents (per cent of fresh weight) in potato tubers, Eva, Bintje, and President varieties, irradiated at about 180,000 rad November 1956 to May 1957 and November 1957 to April 1958. ■, irradiated; □, control.

Fig. 3 shows the computed analytical results of the sugar contents of the three potato varieties, Eva, Bintje, and President; irradiation was carried out from November 1956 to May 1957, and from November 1957 to April 1958, by a mean dose of 180,000 rad. The variations on the different irradiation occasions were negligible (cf. Table 1). It can be seen from the figure that relatively large differences exist

Table 1. The sugar contents (per cent of fresh weight) in γ -irradiated potato tubers (Bintje) and the corresponding values for peels.

I = irradiated tubers and peels, C = non-irradiated controls.

Dose	Irradiation	Date of Analysis	Sucrose %				Fructose %				Glucose %			
			Tubers		Peels		Tubers		Peels		Tubers		Peels	
			I	C	I	C	I	C	I	C	I	C	I	C
16×10^4	6.11.–10.11.56	12.11.56	3.0	0.3			0.1	0.3			0.4	0.2		
18×10^4	17.–21.12.57	8.1.57	3.1	0.3	2.2	0.2	—	0.3	0.1	0.3	0.8	0.4	0.6	0.3
18×10^4	17.–21.12.57	24.4.58	3.5	0.3			0.1	0.4			2.0	1.0		
18×10^4	24.–28.1.58	30.1.58	3.0	0.2	2.0	0.2	0.2	0.4	0.1	0.3	0.5	0.3	0.4	0.3
18×10^4	24.–28.1.58	19.1.58	3.0	0.3	2.4	0.4	0.2	0.4	0.1	0.2	0.6	0.3	0.3	0.2
18×10^4	12.–16.2.58	26.3.58	3.0	0.4	3.0	0.4	0.1	0.8	0.1	0.4	1.0	0.8	0.8	0.5
1.3×10^6	23.3.–23.4.58	24.4.58	3.5	0.2			—	0.3			1.5	0.2		
2×10^6	2.11.–9.12.56	10.12.56	3.5	0.1			—	0.5			0.8	0.5		
2×10^6	2.11.–9.12.56	10.12.58	4.0	^a			—	^a			1.0	^a		

^a The control tubers to this dose, which were stored for two years at +5°C, could not be used for sugar determinations.

Table 2. The effect of variations in the dose-rate of γ - and X-irradiation on sprouting of potato tubers (Eva variety).

The tubers were stored at $+5^{\circ}\text{C}$ and the sprouting was not promoted by elevation of temperature or humidity. The results are given as the average number of sprouts (longer than 1 mm) per tuber.

Dose		Irradiation time min.	Dose-rate r/min.	Average number of sprouts	
γ -rays	X-rays ^a			25.1.1957	30.4.1957
800		1	800	2.9	4.9
	800	1	800	4.8	6.1
	800	25	32	6.4	7.2
1920		60	32	2.8	4.6
	1920	60	32	4.4	4.8
4000		5	800	3.2	4.4
4000		125	32	3.7	4.6
	4000	5	800	3.6	3.9
8000		10	800	1.9	3.1
8000		63	126	2.9	4.3
	8000	10	800	1.9	1.9
	8000	63	126	2.1	2.9
Non-irradiated tubers				4.5	5.7

^a Given by means of a therapy apparatus run at 190 kV; filtering 2 mm Al.

between the sugar compositions of the different varieties. (As to the variations in the sugar composition dependent on potato variety and storage conditions, see for instance Emilsson (8) and Schwimmer *et al.* (9).) It can also be seen from the figure that the effect of irradiation on all three types of test tubers is approximately the same. The mean results for the three varieties were: a sucrose increase of 900 %, a glucose increase of 100 %, but a fructose decrease of 80 %. Irradiation at the two temperatures, $+5^{\circ}\text{C}$, and $+20^{\circ}\text{C}$, gave completely analogous changes in the sugar composition of the tubers; the control tubers, which were stored in the irradiation room, as mentioned previously, gave somewhat lower sugar values when kept at $+20^{\circ}\text{C}$ than when they were kept at $+5^{\circ}\text{C}$.

Observations on inhibition and stimulation of sprouting

With regard to the inhibition of sprouting, both germination analyses and field experiments have shown that doses of, or higher than, 18,000 rad are totally inhibiting, and that doses as low as 2000 rad appear to reduce sprouting. This agrees well with the findings of other authors, e.g. Sparrow and Christensen (4, 5), and Evans (10). A pronounced stimulation of germination was observed at doses of 350–800 rad. Here it should be noted that factors, such as the dose-rate, the time of irradiation (length of time after harvest), and the potato variety, play an important role. Yield-tests with the Bintje and President varieties also gave positive results, i.e. approximately a 15 % higher yield with seedling-tubers, which were irradiated by 400–800 rad. These investigations, which demand a very large material if the statistical values are to be reliable, are still in progress and the results will be published later. As an example of this stimulation, a comparative series with irradiation with γ - and X-rays (Table 2)

Table 3. Results of yield-tests with γ -irradiated potato tubers (Bintje and President varieties).

Potato variety	Dose γ -rays rad	Average number of tubers per plant	Average weight per 100 tubers kg	Mean yield per 20 seedling-tubers kg
Bintje	—	8.8	7.9	10.0
	400	9.8	7.6	16.4
	600	10.7	11.0	11.8
	800	9.3	8.6	10.0
	800	9.1	7.8	10.0
	1,000	8.3	6.0	8.0
	1,000	7.7	8.9	9.2
	2,400	5.0	4.7	4.6
	4,800	3.8	3.8	2.8
	180,000	—	—	—
President	—	7.4	11.3	12.0
	250	6.8	10.8	11.8
	500	5.6	11.5	21.4
	800	6.4	18.3	22.2
	2,000	5.9	8.9	9.0
	4,000	5.9	8.7	8.0
	10,000	4.3	2.7	1.6

is presented here. The importance of the dose-rate, particularly at lower doses and between the time interval of 1–25 min, is obvious. In this test series with the Eva variety 800 rad γ -irradiation (note the high dose-rate) did not cause any stimulation, whereas 800 rad X-rays did give rise to stimulation. Stimulation of the germination was obtained in all three named varieties, Eva, Bintje, and President, from γ -irradiation at very low dose-rates. The preliminary results of yield tests with the Bintje and President varieties are to be found in Table 3.

Discussion

The results stated above indicate that inhibition of the sprouting and changes in the sugar metabolism are two unrelated effects. Whereas a dose of 16,000 rad inhibits germination definitely and almost totally, the changes in the sugar contents of the tubers are reversible. After the present investigation was nearly terminated a paper of Schwimmer *et al.* (11) appeared, where almost similar results with regard to low doses were related. Contrary to the results in the present paper these authors, however, found an increase also in the fructose content. Higher doses were not applied by these authors. The present investigation includes also heavy doses amounting to 18×10^4 , 1.3×10^6 , and 2×10^6 rad. In these dose levels the influence of the irradiation on the sugar metabolism was found to be irreversible and even enzymatic effects were observed (particularly considering potato phosphorylase and Q-enzyme). As to the lower doses, which already inhibited sprouting, no enzymatic changes could be observed.

Another factor which has been studied is the changes in starch content of the tubers. Up to now, it has not been possible to observe any changes induced by

Table 4. pH in the press-juice of irradiated and non-irradiated tubers (Bintje variety) one month and five months after harvest.

	pH values	
	30.10.57	5.5.58
Dose γ -rays, rad		
400	5.85	6.07
2×10^5	5.97	6.14
1.3×10^6		6.57
Non-irradiated		
(1)	5.92	6.18
(2) ^a	5.89	6.11
(3) ^b		5.96

^a Stored five days behind iron-concrete and lead in the ^{60}Co -room.

^b Stored one month behind iron-concrete and lead in the ^{60}Co -room, i.e. during the irradiation period at the dose 1.3×10^6 rad.

irradiation, but it should be pointed out that the available methods applied were not found to be reliable. An *in vitro* investigation of the influence of irradiation on starch and other polysaccharides (12) has shown that potato starch becomes soluble at doses of about 100,000 rad and that solutions of the irradiated starch are acidic. However, no such changes in the starch of the irradiated tubers was observed, nor was the pH value of the press-juice from irradiated tubers lower than the pH value in the press-juice of non-irradiated tubers (see Table 4). On the contrary, the pH at the dose level 1.3×10^6 rad was somewhat higher than the pH in the corresponding control. This is probably explained by the protective effect of water and other compounds against irradiation.

Porter and Edelman (13) have investigated the sucrose metabolism in sections of tobacco leaves with the help of radio-active labelled sugar. These authors conclude that sucrose is perhaps not on the direct pathway of starch synthesis, but that the link between carbon dioxide and starch is effected through sugar derivatives, possibly phosphate esters. Sussman (14) has studied the effect of ionizing radiations upon the respiration and the oxidases of the potato tuber. He found that the evolution of carbon dioxide is stimulated in irradiated tubers, and Schwimmer *et al.* (11) stated that changes in sucrose content of irradiated tubers kept at 70°F ($+21^\circ\text{C}$) follow the same curves. Sussman, irradiating the tubers with doses from 100 r to 3.2×10^6 r, reported also an up to 600 % increase in Q_{O_2} . The oxidase activities were not affected by the dosages used, except that the tyrosinase lost half of its activity at 3.2×10^6 r. In the present investigation it was found that the surface of press-juice from irradiated tubers, particularly at higher doses (1.3×10^6), darkened sooner than that of the control juice. The darkening could perhaps be due to enzymatically catalyzed oxidations leading to melanin formation. According to Mason (15) the initial oxidation of tyrosine or 3,4-dioxyphenylalanine is effected by tyrosinase or by the related "dopa" oxidase. An examination of the amino acid contents in irradiated and control tubers was therefore performed, but no radiation influence upon tyrosine or 3,4-dioxyphenylalanine could be stated; an increase in the serine content at the dose level 2×10^6 rad was, however, noted.

The changes in the sugar metabolism of the potato tuber might be caused by the same factors causing changes in the sugar composition in *Vicia faba* (1), although these factors have not yet been identified. It is, however, possible that quite different systems in the potato tubers are influenced. A closer investigation of the enzymes already found in the tubers, as well as those, which might occur in extremely small quantities and have not yet been identified, would probably provide the answer to this question.

Leloir *et al.* (16, 17) have shown, in a number of different plant species, enzymes which catalyze the reactions:

- 1)
$$\text{F-6-P} + \text{H}_2\text{O} \rightleftharpoons \text{Fructose} + \text{P}$$
- 2)
$$\text{UDPG} + \text{Fructose} \rightleftharpoons \text{Sucrose} + \text{UDP}$$
- 3)
$$\text{UDPG} + \text{F-6-P} \rightleftharpoons \text{Sucrosephosphate} + \text{UDP}$$
- 4)
$$\text{Sucrosephosphate} \rightleftharpoons \text{Sucrose} + \text{P}$$

where UDP = uridine diphosphate, UDPG = uridine diphosphate glucose, and F-6-P = fructose-6-phosphate.

It is possible that enzymic systems, or intermediate links, which catalyze the above reactions, are influenced by the irradiation. Even changes in the ATP-balance (adenosine triphosphatase has been found in potato tubers) can be the indirect cause of the changes in the sugar metabolism. This assumption lies close to hand as other investigations in this series (18) have shown that the phosphate metabolism in the tubers is also influenced by irradiation.

Regarding stimulatory effects of irradiation in other plants, the earlier literature reports contradictory results. Some authors have found increased germinability, others have not (cf., for instance, Johnson (19) and Ehrenberg (20)). The results of the present investigation indicate, however, that ionizing radiation by small doses and at low dose rates have a stimulating effect on the germination of potato tubers.

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The reaction of extremely basic chromium perchlorates with proteins

I. Intact and modified collagen and polyamides

By K. H. GUSTAVSON

According to the generally accepted view, the irreversible fixation of positively charged chromium complexes by collagen involves its charged carboxyl groups, which are discharged by the cationic complexes (1). The final result is formation of an exceedingly stable coordinative-covalent link between the chromium atom and the carboxyl group. A small part of the fixed polynuclear chromium complexes is attached to at least two carboxylic groups on adjacent polypeptide chains, forming a stable cross-link (1, 2, 3). Such intermolecular links stabilize the protein and are responsible for the tanning effect (1). The basic chromium sulphates are of technical importance in chrome tanning and have therefore been most thoroughly studied, while the basic chromium chlorides are chiefly of theoretical interest (4). In equilibrated dilute solutions of basic chlorides, of composition corresponding to the empirical formula $\text{Cr}_2(\text{OH})_n\text{Cl}_{6-n}$, where $n \leq 2$, and those of basic sulphates corresponding to the formula $\text{Cr}_2(\text{OH})_2(\text{SO}_4)_2$, or with one OH groups per each atom of chromium at the most, cationic chromium complexes preponderate (90–100 % of the total chromium; the figure for the chlorides being 100 %) (5).

By introducing a greater number of hydroxy groups per atom of chromium, for instance by the addition of alkali to reach compounds of composition corresponding to the empirical formula $\text{Cr}_2(\text{OH})_4\text{Cl}_2$ (the 33 % acid salt according to the present nomenclature), considerable amounts of uncharged chromium complexes are formed as indicated by electrophoresis (6), ion exchange (5) and methods of preferential sorption (6). The solutions of these extremely basic chlorides show in their reaction with collagen a more complicated picture than the cationic compounds. Apart from the ordinary type of reaction of their cationic chromium complexes with the carboxyls, the major fixation of chromium in the form of uncharged complexes involves non-ionic protein groups, such as the keto-imide and hydroxy groups of the hide protein (7). By investigating variously modified specimens of collagen, certain indications of the binding sites on the protein for the reactants are obtainable (8). The inactivation of carboxylic groups by esterification, the ϵ -amino groups of lysine by diazotization and the hydroxy groups by acetylation yield typical modified collagens which have proved useful for differentiating the ionic and non-ionic reactions of various agents with collagen.

In the present investigation, some aspects of the reaction of extremely basic perchlorates of chromium with collagen proper, and with variously modified speci-

mens, have been studied. No experimental study of the interaction of aqueous solutions of perchlorates of chromium with proteins is recorded in the literature. Only very occasionally has some data on these systems been forthcoming in connexion with the present author's investigations (9, 10) of other major chromium salts, although their behaviour to collagen has been long comprehensively studied (11). Actually, the extremely basic compounds of these salts offer exceedingly interesting reactions; even an unique type of fixation has been discovered. From the physico-chemical point of view, the perchlorate anion is important and frequently introduced in studies of complex formation, electrical conductivity etc., of heavy metal salts in aqueous solution, since this anion is not itself complex-forming. However, under certain conditions, for instance in solutions of extremely basic chromium perchlorates, the perchlorate anion may function as a complexing agent.

The ClO_4 group in combination with chromium behaves like the Cl group, as far as the composition of the extremely basic chromium compounds is concerned, and also in relation to behaviour on ion exchange and electrophoresis, and its reactivity with and affinity for proteins (11).

Experimental

Basic chromium perchlorates

Solutions of the 67 % acid chromium perchlorate of composition corresponding to the empirical formula: $\text{Cr}_2(\text{OH})_2(\text{ClO}_4)_4$ were prepared by dissolving freshly precipitated hydrous chromic oxide $\text{Cr}(\text{OH})_3$ (obtained by adding ammonia to a solution of the hexaquo-chromium chloride) in the required amount of perchloric acid (72 % HClO_4) with warming. After keeping the solution at 90°C for 10 minutes, it was diluted to 4.0 eq./l Cr, and aged for at least 8 weeks before use. The extremely basic chromium perchlorate was prepared by adding a 10 % solution of sodium hydroxide to the aforementioned standard solution in a quantity of one equivalent of NaOH per atom of chromium. After heating to 90°C , the solution was diluted to contain 1.00 eq./Cr, and aged 8 weeks before use. Its composition was close to the formula: $\text{Cr}_2(\text{OH})_4\text{Cl}_2 \cdot 2\text{NaClO}_4$. Its % acidity was 28.

On analysis of this standard solution by the ion exchange method (5), employing a column with Dowex-50 resin, operated in the sodium cycle, 76 % of the total chromium was found to be present in the form of non-cationic complexes (uncharged) and the rest, 24 %, as electropositive chromium complexes. On percolating the solution through a column of anion exchange resin of the quaternary base type (Amberlite IRA-400), operating in the form of the nitrate, all the chromium was found in the filtrate, proving the absence of anionic chromium. The composition of the filtrate from the cation exchanger, estimated by analysis of the contents of Cr and hydrolyzable perchlorate, corresponded to the 17 % acid perchlorate, represented by the empirical formula: $(\text{Cr}_2(\text{OH})_5\text{ClO}_4)_n$. The composition of the rest of the solution which consisted of cationic chromium, fixed by the cation exchanger, was obtained indirectly. The acidity was 64 %, corresponding closely to the compound: $\text{Cr}_2(\text{OH})_2(\text{ClO}_4)_4$. Electrophoresis of the solution of the 28 % acid chromium perchlorate showed about one third of the total chromium migrating as positively charged complexes, the rest moving very slowly or not at all. On paper electrophoresis (6), about one fourth of the total chromium moved normally, while the main part remained in the spot as applied, indicating an uncharged state of the complexes. In view of the com-

plication likely to occur by absorption of the extremely basic fraction of the chromium compounds by the paper in this method of ionophoresis, no quantitative estimation of the various fractions was attempted. Estimation of the average molecular weight of the extremely basic fraction of the corresponding chromium chloride by diffusion (6), employing the McBain-Northrop-Anson technique (12, 13) with fritted glass filters, showed values of the order of 800–1000 (6), indicating the main component to contain 6 chromium atoms per molecule. Hence, the aqueous solutions of the extremely basic perchlorates of chromium, with acidities in the range 28–33 %, are indicated by the present data to consist of mixtures of the $(\text{Cr}_2(\text{OH})_5(\text{ClO}_4)_n$ compound and the $(\text{Cr}_2(\text{OH})_2(\text{ClO}_4)_4$ compound; the former being uncharged, the latter entirely in the cationic form.

In the present investigation, data on the behaviour of the purely cationic type of chromium perchlorates were needed and are included for comparison, and for the sake of demonstrating differences in reaction towards the various substrates. An equilibrated solution of the 67 % acid chromium perchlorate, corresponding to the formula: $\text{Cr}_2(\text{OH})_2(\text{ClO}_4)_4 \cdot 2\text{NaClO}_4$, containing the same amount of sodium perchlorate as the extremely basic compound, was prepared by adding one equivalent of sodium hydroxide to each atom of chromium present in the form of the hexaquo-chromium perchlorate. This, in its turn was obtained by dissolving freshly precipitated hydrous chromic oxide (from $\text{Cr}(\text{OH})_2\text{Cl}_3$) in the stoichiometric amount of boiling perchloric acid. The final aged solution, at a concentration of one eq. Cr per liter, consisted of cationic chromium complexes exclusively.

Modified Collagens

Isoelectric collagen ($\text{P}_I:5.3$) in the form of hide powder, containing less than 0.1% ash and with an N-content of 18.02 %, served as the main substrate (denoted Blank in the tables). A specimen of esterified hide powder, methylated according to the procedure of Fraenkel-Conrat and Olcott (14) as modified and described by the author (15), represented collagen with its carboxyl groups almost completely inactivated (16) (0.80–0.85 meq. esterified carboxyl groups/g collagen). The participation of the ϵ -amino groups of the lysine residues in ionic reactions is an important aspect of collagen reactions. Hence, a deaminated (diazotized) hide powder specimen, with 0.36 meq. N/g collagen removed, as described earlier (17, 18), was employed for evaluation of the effect of the intact ϵ -amino groups on the chrome fixation. Further, hide powder with its ϵ -amino groups (0.39 meq./g collagen) completely acetylated, N-acetylated, according to the procedure of Green *et al.* (19, 20) and a specimen exhaustively acetylated, i.e., with the amino groups completely acetylated and about three fourth of the total number of the hydroxy groups inactivated by the $-\text{CO} \cdot \text{CH}_3$ group (N- and O-acetylated) were included in the series of substrates. (For preparation, see (20)). The exhaustively acetylated collagen contained 1.70 meq. acetyl/g collagen.

In view of the fact that hydrothermal denaturation (shrinkage) of collagen changes its reactivity, particularly that of the non-ionic groups which are of importance in coordination reactions (21), hide powder denatured in 70°C water for one minute was included among the substrates, as was also hide powder which had received a pretreatment with a concentrated solution of a lyotropic agent, 2 *M* solution of sodium perchlorate, for 7 days at 20°C (22). The destruction (solubilization) of the hide powder (1 g of collagen per 20 ml of the 2 *M* solution) amounted to 36 % of the original weight of the hide protein.

Solutions of sodium periodate at moderate temperatures (20°C) showed the same type of action. Apart from the well-known effect of oxidation and splitting of the



link (α -glycols (23–25)), periodate in solutions of 0.25–0.5 *M* strength is an active hydrogen-bond breaking agent, increasing substantially the coordination capacity of collagen (26). Finally, three specimens of pretanned collagen were employed. By completely inactivating the ionic protein groups (the carboxyls as well as the total basic ones (lysines + guanidyl)) with irreversibly fixed condensed polymethylene-naphthalene-disulphonic acid (26.4 % fixed sulpho-acid, on collagen basis) according to a method described earlier (27), the ionic reactivity of collagen is entirely eliminated, e.g., the uptake of 0.1 *N* HCl. The coordinate reactivity of the non-ionic groups, primarily the $\text{—CO}\cdot\text{NH—}$ links and the hydroxy groups of collagen, is not altered by this treatment. Hence, this substrate would be expected to be useful for evaluating the role of the non-ionic protein groups. Finally, two types of vegetable tanned specimens of hide powder close the list of modified substrates; the one tanned thoroughly with tannic acid, to an amount of 91 % irreversibly fixed tannin, contained a great part of the irreversibly fixed tannins firmly attached to the keto-imide groups of collagen; while the specimen tanned with mimosa extract, to contain 76 % irreversibly fixed tannin on the basis of collagen also carried a large part of the irreversibly fixed polyphenols firmly bound to the basic protein groups. In both instances, the coordinate reactivity of the $\text{—CO}\cdot\text{NH—}$ groups is fairly well satisfied and also a large part of the valency function of the basic protein groups (28).

Polyamides

It was deemed advisable to include in this study a substrate containing the keto-imide link as the main reactive group, but devoid of electrovalent groups, or nearly so. Modified Nylons, such as certain Polyamides, meet these requirements (9, 29, 30, 31). It is important that a part of the $\text{—CO}\cdot\text{NH—}$ groups are not intermolecularly compensated by H-bridging; also that the interstices are large enough to accomodate medium-sized molecules. In fibers of too strongly developed intermolecular linking of the individual chains, e.g., the regular polyamides, such as Nylon of the original type, the chemical inertness of the polyamide is the result of (i) the non-availability of coordinate loci on the compensated peptide groups of the tightly aligned molecular chains, and (ii) the lack of intermolecular and interfibrillar space. The latter implies steric hindrance. The monotony of the repeating units of drawn Nylon fibre, in its original form $\text{—CO}\cdot\text{NH}\cdot(\text{CH}_2)_6\text{NH}\cdot\text{CO}(\text{CH}_2)_4\cdot\text{CO—}$ structures, makes for compactness, insolubility and chemical inertness. If bulky side chains are introduced, a certain degree of irregularity is imparted to the chains.

A polyamide of the copolymer type was obviously needed for the present work. Such a polyamide, *Igamid 6A*,¹ was employed to evaluate the extent of chrome fixation by the keto-imide groups (29, 30). It is a copolymer of the adipic acid-hexamethylene-diamine salt (60 %) and caprolactam (2-oxo-hexamethylene-imine (40 %), (29)). The total nitrogen content was 12.4 %, on the weight of the dry substance. The polyamide was brought into a more reactive form by dissolving it in

¹ A commercial product of the *Badische Anilin & Soda Fabrik*, Ludwigshafen, Germany. A sample generously furnished by Professor H. Hopff, Ludwigshafen, in 1950 is gratefully acknowledged.

boiling methanol (3.3 % solution). This solution was then stirred into cold water in which the polyamide was precipitated in the form of white woolly fibres. The hydrated powder was washed, and mechanically adhering water removed by suction. The hydrated polyamide contained 25 % dry substance.

The hydrated polyamide showed the following characteristics: 12.4 % N, 0.0 % ash. The HCl-binding capacity at the final pH of 1.0 was 0.05 meq. H^+ , and the base binding capacity at the final pH of 12.6 was 0.02 meq. OH^- per g polyamide. The only reactive groups besides the keto-imide group are the terminal carboxyl- and amino-groups, amounting to 0.07 meq. per g polyamide. The electrovalent groups are thus only 3 % of the corresponding groups of collagen, which amount to 2.1 meq. per g protein (32). It should be noted that on dissolving the polyamide in methanol, part of the crosslinking hydrogen bonds on the $-CO \cdot NH-$ links are ruptured. In the subsequent precipitation in water, hydration of the non-compensated $-CO \cdot NH-$ groups takes place.

Interaction

In the standard method of reacting the solutions of chromium perchlorates with the substrates, portions of hydrated specimens equal to 2.0 g collagen (or polyamide) were treated under constant agitation for periods of time from 96 to 144 hrs. with the solution of the perchlorates in a total volume of 50 ml, containing 1.0 eq. Cr per liter, ensuring complete interaction. After separating the treated substrates from the solution of the chromium salt by suction filtering, followed by washing, they were drummed and filtered until free from soluble chromium compounds. The residual solution and the washings in the polyamide series were collected and the contents of chromium and hydrolyzable perchlorate estimated in the collected filtrates. In all instances, the dried chromed substrates were analyzed for their contents of ash, fixed chromium, hydrolyzable perchlorate and nitrogen (protein). For collagen the factor was $N \times 5.56$, and for the polyamide $N \times 8.07$. Additional series were run in several instances to determine the distribution of the perchlorate groups in the substrate, particularly to ascertain the ClO_4 groups present in electrovalent attachment with the protein and the portion of these groups bound in the fixed chromium complexes. By treating the tanned specimens in 50 ml of 4.0 w/v % solution of Pyridine (pH 8.1) for one hour (33), the perchlorate present in the ionic form, compensated by the cationic protein groups and by the fixed chromium complexes, possessing surplus positive charge (counter-ions), is removed.

Results

One hour's treatment of 1.0 g collagen in the form of the chromed specimen No. 1 of Table 1 in 50 ml of 4.0 % Pyridine solution (33), for removal of ionic perchlorate groups left an amount of 0.56 meq. ClO_4 per g collagen resisting the treatment. This implies that the average acidity of the fixed chromium compounds is 11 %. Since about three quarters of the fixed chromium should be in the form of non-ionic chromium complexes, and the cationic chromium is free from complexly bound ClO_4 groups, the acidity of the former complexes is actually 15 %, or not far from the acidity percentage of the compound $Cr_2(OH)_5ClO_4$, which is 16.7.

Table 1. The fixation of chromium perchlorates by collagen and modified collagen.

In meq., fixed by 1 g collagen.

No.	Type of substrate	28 % acid chromium perchlorate			67 % acid chromium perchlorate		
		Cr	ClO ₄	% acidity of fixed Cr compound	Cr	ClO ₄	% acidity of fixed Cr compound
1	Blank H.P.	4.90	1.27	26	2.78	1.30	47
2	Denatured H.P.	9.87	1.88	19	2.81	1.27	45
3	H.P. pretreated with 2 M NaClO ₄ solution	9.98	1.92	19	3.07	1.38	45
4	Deaminated H.P.	5.01	1.10	22	2.53	1.16	46
5	Esterified H.P.	3.69	0.74	20	0.14	—	—
6	N-acetylated H.P.	5.44	1.41	26	2.51	1.20	48
7	N- and O-acetylated H.P.	7.33	1.30	18	3.19	1.50	47
8	Tannin-tanned H.P.	0.28	—	—	0.59	—	—
9	Mimosa-tanned H.P.	0.34	—	—	0.47	—	—
10	Polynaphthalenemethylene sulphonie acid inactivated H.P.	2.95	—	—	1.02	—	—
11	Na-periodate pretreated corium (calf skin)	4.68	1.03	22	2.85	1.41	49
12	Corium (calf skin)	3.45	0.87	25	2.68	1.34	50

Discussion

Modified collagens

The salient points of the data in Table 1 are as follows. By hydrothermal denaturation (shrinkage) of hide powder collagen (No. 2), the reactions occurring at its ionic groups are not affected appreciably (21, 22, 34). Only the non-ionic reactivity of the collagen is altered, particularly the irreversible binding of coordinatively active agents on the sites for hydrogen bonding, i.e., $-\text{CO}\cdot\text{NH}-$ links present in interchain compensation (cross-linked) in the intact collagen, adjacent keto-imide groups on different chains (21, 34) or the hydroxy group of the hydroxyproline residue with the carbonyl group of the keto-imide link (35). This well-established fact (34) is confirmed by the data in Table 1, showing that the reaction of the straight cationic chromium perchlorate is independent of the alterations of the collagen resulting from denaturation, while the fixation of the extremely basic perchlorate, with non-ionic complexes dominating, is more than doubled by the creation of new sites for its coordination on the protein due to the heat-shrinkage process. Similar alterations are imparted to the collagen structure by its pretreatment in the concentrated solution of the effective hydrogen-bond breaking agent sodium perchlorate (22). However, the drastic effect of this neutral salt is not confined solely to the non-ionic groups. Secondary reactions are taking place concurrently, increasing the acidic and basic protein groups to a minor extent, probably by cleavage of some $-\text{CO}\cdot\text{NH}-$ links, as evident from the augmented fixation of the straight cationic type of the chromium perchlorate. This trend of the data for the fixation of the chrome by the denatured collagen from the solution with uncharged chromium complexes as the main constituent, is in harmony

with the concept that the binding of the non-ionic complexes is regulated principally by the non-ionic valency forces on the keto-imide groups.

NH-

By the rupture of the $\text{-OH}\cdots\text{OC-}$ crosslinks (35), a greater number of hydroxyproline-hydroxy groups are made available by the thermal denaturation. However, they do not enter appreciably as a factor, in view of the fact that nearly complete acetylation of the reactive hydroxy groups (No. 7) does not impair but does favour the chrome uptake from the solution of the extremely basic perchlorate. The increased chrome fixation by the N- and O-acetylation of the hide powder from the purely cationic type of perchlorate also indicates that the severe experimental conditions which must be applied for exhaustive acetylation induce a definite increase in acidic protein groups. Evidently, this exhaustive acetylation, as also the esterification of collagen, to be discussed next, alter the reactivity of several types of protein groups in opposite directions, and this makes evaluation of the data difficult. Thus, while the concomitant swelling frees some compensated $\text{-CO}\cdot\text{NH-}$ groups by dislocating

NH-

the hydrogen bond cross-linkages, the breaking of the $\text{-OH}\cdots\text{OC-}$ link by acetylation of the hydroxy group of the hydroxyproline residue results in an increase in coordination sites on the freed keto-imide group, whereas the OH group is inactivated (35). The aforementioned increase of the ionic protein groups adds further to the complexities (36).

The inactivation of the carboxyl groups of the collagen by methylation (esterification) almost completely prevents the reaction of the cationic chromium complexes with the protein. On the other hand, as would be expected, the non-ionic component of the 28 % acid chromium perchlorate possesses marked affinity for the carboxyl-inactivated collagen, about three quarters of the amount bound by the intact collagen being recorded. The exceedingly great degree of swelling of the hide powder, taking place in the methylation at pH 1, leads to permanent dislocation of some cross-links and the formation of new coordination active centres in the esterified collagen (37). Comparing the figures of meq. chromium fixed by the intact and esterified specimens, the proportions correspond closely to the ratio of the amounts of total chromium to the non-ionic chromium. However, this is probably a mere coincidence. These data are of interest, since they prove the participation of types of fixation of chromium other than the ordinary chrome binding by the carboxyl ions of collagen, in the instance of systems containing uncharged chromium, or non-cationic chromium complexes.

In order to broaden the experimental basis for the concept postulating that the mechanism of reaction of the non-ionic chromium complexes involves the participation of the keto-imide groups of collagen for irreversible binding, tanned specimens of collagen were investigated. It has been established definitely that the main part of the vegetable tannins combine with the collagen through $\text{-CO}\cdot\text{NH-}$ groups. One of the most convincing evidences is that polyamides, with the keto-imide group as the exclusive reaction centre, bind tannins irreversibly in large quantities, a striking similarity to the behaviour of collagen (29, 30). Thus, the specimen of hide powder tanned heavily with tannic acid has practically all its keto-imide groups blocked, and this also applies to the mimosa tanned hide powder. The condensed polyphenols of this type of vegetable tannin also form firm bonds with the basic protein groups.

The data on the chromium fixation by these specimens (Nos. 8 and 9) prove their lack of affinity for the chromium compounds, especially for the extremely basic perchlorate.

In specimen No. 10, the ionic groups of the collagen have been permanently inactivated by the irreversible fixation of the condensed sulphonic acid (a polynaphthalene-methylene-sulphonic acid) (38). This acid reacts specifically with the electrovalent protein groups. Thus, it does not interfere with the reactivity of the non-ionic protein groups noticeably. The great majority of the keto-imide groups of the intact collagen should thus not be affected by the blocking. It is known (11) that the fixed sulphonic acid binds chromium from solutions of ordinary cationic chromium compounds, particularly so the basic chromium chlorides. The reason is the great complexing power of the sulphonic acid group, as compared to the ClO_4 -group. Direct chrome binding on the protein by displacement of the sulpho-acid adds further complication. The uptake of strictly cationic chromium complexes by the modified collagen which is devoid of ionic reactivity is thus explained. Even though these particular data do not allow of quantitative treatment, they prove without any doubt that for the heavy fixation of the extremely basic perchlorates, non-ionic reaction centres of the collagen are participating and governing.

As to the function of the cationic protein groups which are indirectly concerned in the binding of cationic chromium complexes by collagen by virtue of their function as compensator of the anions of the chromium salts, a reaction intimately linked up with the primary reaction of the charged carboxyl groups (39, 40), the specimens Nos. 4, 6 and 7 are of interest, als already mentioned. By the deamination and the N-acetylation of the collagen, the ϵ -amino groups of the lysine residues are inactivated, as far as their ionic function goes. Such modified collagen shows a slightly diminished uptake of cationic chromium (36, 39), due to the lowered acid binding capacity, which in its turn retroacts on the fixation of the corresponding cationic chromium complexes by the carboxylic ions, an indirect effect (39). However, the fixation of uncharged chromium complexes by collagen from the solution of the extremely basic perchlorate of chromium is moderately increased by the deamination and N-acetylation processes. In both instances, light swelling of the collagen takes place, resulting in permanent alteration of the cohesive forces of the structure, indicated by earlier investigations to be due to the rupture of intermolecular stabilizing links (10, 37). While the fixation of the portion of the cationic chromium compounds in the 28 % acid perchlorate should be lowered as a result of the interrelationship of the discharge of the anions of the chromium salt and the chromium cations by the modified protein, the augmented binding of the non-ionic constituents of the chromium compound is expected to outbalance this negative effect of the inactivation of the basic protein groups on the cationic chrome fixation, the sum total being increased chrome uptake by the modified collagen.

With regard to the data on substrate No. 7, the exhaustively acetylated hide powder with 80 % of its hydroxy groups inactivated, the following points should be noted. This acetylation, in contrast to the simpe N-acetylation lowers the hydrothermal stability as measured by the shrinkage temperature, T_s , by 20–25°C, proving severe weakening of the intermolecular cohesion (35). The chrome fixation is increased in both instances, very markedly so for the non-ionic type of chromium complexes. As mentioned earlier, any conclusion as to the role played by the hydroxy groups in the chrome uptake cannot safely be drawn. Generally, any method applied to modify a particular group of the protein which concomitantly deeply alters the non-ionic

Table 2. The fixation of chromium perchlorates by polyamide.

In meq., fixed by 1 g polyamide.

	28 % acid chromium perchlorate	67 % acid chromium perchlorate
Cr	3.67	0.12
ClO ₄	0.60	0.06
% acidity of fixed Cr complexes	16	50

protein groups and the cohesion of the substrate, must be applied cautiously in systems containing variously charged and uncharged chromium complexes, particularly those of a high degree of condensation (large polynuclear complexes). This drawback is also prominent in the case of the hide powder which has been pretreated in the concentrated solutions of periodate, which under the conditions employed mainly exerts lyotropic effects. Apart from oxidation of any smaller amounts of carbohydrates associated with collagen, the only residue of the collagen which is severely attacked appears to be the hydroxylysine residue (26) which, present in an amount corresponding to 0.08 meq. per g collagen (32), possesses the particular carbon to carbon link most easily cleaved by the periodate, i.e., the $-\text{CH}-\text{CH}_2$ link (46).



Polyamides

The conclusive proof of the thesis advanced for the non-ionic chrome tannage is supplied by the data in Table 2. The polyamide relies practically completely on its free coordination sites on the non-compensated $-\text{CO}\cdot\text{NH}-$ groups for its reactivity, as shown, for instance, by the lack of affinity of the polyamide for cationic chromium complexes (9). Large amounts of chromium, 3–4 meq. Cr per g polyamide, or in terms of Cr_2O_3 7–10 % Cr_2O_3 on the polyamide content, are irreversibly fixed. For the extremely basic perchlorates of chromium the chrome fixation amounts to about three quarters of the amount fixed by collagen from the same solution. By the "by difference" method, i.e., from analysis of the original and residual solutions for Cr and hydrolyzable perchlorate, the composition of the fixed chromium compounds was indirectly obtained. It proved to be 17 % acid, corresponding to the empirical formula: $(\text{Cr}_2(\text{OH})_5\text{ClO}_4)$. By direct analysis of the chromed polyamide, the acidity of the complex attached irreversibly to the amide-substrate was 16 %, in excellent agreement with the indirectly obtained figure. This finding suggests that the solution of the 28 % acid chromium perchlorate studied is mainly composed of about one mole each of $(\text{Cr}_2(\text{OH})_5\text{ClO}_4)_3$ and of $\text{Cr}_2(\text{OH})_2(\text{ClO}_4)_4$ (41). The acidity of the residual solution after complete removal of the uncharged complexes by the polyamide was 62–68 %, while the acidity of the cationic component mentioned is 67 %.

Hydrothermal stability

Finally, it should be noted that by tanning whole calf-skin pieces with the extremely basic chromium perchlorates, amounts of 3–5 meq. Cr per g of collagen

were introduced. After removal of the ionic ClO_4 groups from the chromed skin by the pyridine method (33), the shrinkage temperature, T_s , was in the range 83–87°C, or an increase of the T_s of intact collagen of an order of 20–25°C. The corresponding value for the ordinary cationic tannage with basic chromium sulphates exceeds 50°C generally (42, 43). The degree of stabilization of the collagen lattice effected by the non-ionic chrome tannage is of the same order as that found for the most effective vegetable tannins, which interact with the keto-imide groups principally under hydrogen bonding of the phenolic groups and partial cross-linking (44).

The participation of the $-\text{CO}\cdot\text{NH}-$ group in complexing and binding of metal complexes has recently been shown to occur with Cu(II) ions on the tripeptide of glycine also, involving proton displacement on the imide groups (45).

Further theorization will be postponed and reserved for a forthcoming paper which will contain results from experiments with the extremely basic chromium perchlorate reacting with gelatin (precipitation) and various ion exchangers in systems with solution-substrate proportions of the same order as those described in this paper. These findings, complemented with data on the reactivity of collagen in combination with these non-ionic chromium complexes towards vegetable tannins, dyestuffs and some special agents, as well as the isoelectric point of the non-cationic chromium collagen compounds conform to and support the concept outlined in this paper.

SUMMARY

The reaction of solutions of extremely basic chromium perchlorate with intact collagen and with specimens of collagen with selected reactive groups modified or inactivated (carboxyl, ϵ -amino, hydroxy, total ionic and keto-imide groups) has been investigated with a view to ascertaining the nature of the irreversible fixation by collagen of the non-cationic part of the chromium complexes present in these solutions. These uncharged complexes are the main constituent of these solutions.

The extent of chrome fixation by the various substrates conforms to the concept of the main reaction being located at the *keto-imide groups* of the hide protein. The non-ionic complexes fixed by a modified polyamide, reacting exclusively by valency centres on the $-\text{CO}\cdot\text{NH}-$ groups, amount to about 70 % of the amount of chromium fixed from the extremely basic perchlorate by intact collagen. The average composition of the fixed complexes corresponds to the empirical formula: $(\text{Cr}_2(\text{OH})_5\text{ClO}_4)_n$. This type of chrome fixation is definitely inferior to the ordinary type of cationic chrome fixation by collagen as far as the hydrothermal stabilization of the collagen lattice is concerned, as proved by the data on the shrinkage temperature, T_s ; the non-ionic chrome tannage raising the T_s 20–25°C, or to about the same extent as do the most potent vegetable tannins which also react specifically with the keto-imide groups. It is noteworthy that the ordinary cationic chrome tannage with basic chromium sulphates normally gives T_s increases greater than 50°C.

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Blood volume and ^{59}Fe distribution in TE mice bearing Ehrlich ascites carcinoma growing in the solid form

By GÜNTER V. EHRENSTEIN

With 2 figures in the text

It is well known that a neoplastic disease is often associated with an anaemia which is not due to blood loss, infection, iron deficiency, nutritional factors or overt haemolytic anaemia.

In the past years numerous communications have appeared on the pathogenesis of this type of anaemia leading to the following conclusions (1–13):

The shortening of the erythrocyte life span and the increased rate of erythrocyte destruction lead to an increased rate of erythropoiesis, which is not adequate to compensate completely for the increased rate of erythrocyte destruction and thus anaemia develops. The corresponding need for iron for the formation of new haemoglobin is, to a large extent, responsible for the increased iron turnover.

Having demonstrated that the life span of the red blood corpuscles of TE mice bearing Ehrlich ascites carcinoma growing in the solid form is shortened (14), the problem arises, if and to what extent the bone marrow of the cancerous mice is able to compensate for the shortened life span and the correspondingly increased rate of destruction of the erythrocytes.

Another problem which arises is whether there is a connection between the enlargement of the spleen and liver (23), and the increased destruction of red blood corpuscles, and also if an extramedullary erythropoiesis takes place in these organs.

In order to solve the first problem, we had to ascertain the extent of anaemia. To arrive at this magnitude we determined the total circulating red corpuscle mass and the haemoglobin concentration of the blood.

To approach the second problem, we compared the uptake of radioiron by the spleen and the liver of cancerous and of control mice at different times after the injection of radioiron, making use of the method first applied by Huff *et al.* (15, 16).

In the present communication, studies on the blood volume, the circulating red corpuscle mass, the total circulating haemoglobin, and the distribution of radioiron in the organs of TE mice bearing Ehrlich ascites carcinoma growing in the solid form, are reported.

Material and methods

Male TE mice, kindly supplied by the Animal Department of the British Medical Council, were used in this investigation. Their average body weight was 30 g and they were kept on a standard diet.

The tumour was an Ehrlich ascites carcinoma kindly given to us by Prof. G. Klein. 2 to 6×10^6 cells suspended in saline were injected intramuscularly into the right hind leg of each mouse. Progressive tumour growth occurred in all animals. Tissue examination revealed no metastases. No bleeding occurred except a short time before death, when the tumour was necrotic and ulcerated.

The animals were bled from the retroocular sinus under Nembutal narcosis. 1–1.5 ml blood were obtained from each animal. Heparin was used as anticoagulant, if not stated otherwise.

Haematological studies were carried out according to standard methods.

The haemoglobin was determined as alkaline oxyhaemoglobin by the method of Evelyn (17) in a Beckman spectrophotometer.

The tissue iron determination was carried out by the sulphosalicylic acid method of Lorber (18), after wet ashing of the samples by the method of Kahane (19).

Radioiron studies were carried out applying ^{59}Fe with a specific activity of 2 microcuries per microgram. 1–4 microcuries ^{59}Fe in 3.8 per cent (w/v) ammonium-citrate solution were injected intraperitoneally into each animal.

The fractionation of the spleens and the livers, and the isolation of ferritin and haemosiderin is discussed in a paper by the author and Lockner (20).

The determination of the circulating red corpuscle mass was carried out applying erythrocytes of healthy donor mice, either labelled *in vitro* with ^{51}Cr as sodium chromate according to Gray and Sterling (21), or labelled *in vivo* by injecting donor mice intraperitoneally with 4 microcurie ^{59}Fe as ammonium-citrate 10 days prior to removing the blood. ACD solution was used as anticoagulant. The tagged erythrocytes were washed three times with saline and resuspended in saline. 0.1 ml of this suspension was injected into a tail vein of the receptor animals. The animals were bled 15–30 minutes after injection of the erythrocytes. The red corpuscles were spun down, washed three times with saline and, after weighing, the radioactivity of the packed red corpuscles was compared with the radioactivity of a standard sample. The blood volume was calculated from the venous haematocrit values corrected by the factor 0.88 (22).

The total circulating haemoglobin was calculated from the haemoglobin concentration and the blood volume.

We used a scintillation counter in the determination of ^{59}Fe and ^{51}Cr . All our liquid samples had a volume of 10 ml. Blood corpuscle samples were haemolyzed with distilled water prior to determining their radioactivity. The counting error was ± 1 per cent.

Results

Blood volume studies

The total circulating red corpuscle mass, the blood volume and the total circulating haemoglobin of healthy and tumour bearing mice are shown in Tables 1 and 2. The animals were killed 21 days after tumour transplantation. The average tumour weight was 1.5 g. The body weight before tumour inoculation was 30 g.

The blood volume of the cancerous animals is significantly higher than that of the controls, due to an increase of the plasma volume, which is reflected by the low haematocrit figure, the low number of erythrocytes per unit volume and the low haemoglobin concentration.

Table 1. Blood volume, total circulating red corpuscle mass, and total circulating haemoglobin of healthy male TE mice.^a

Body haematocrit ^b %	Number of red corpuscles × 10 ⁶ per mm ³	Haemoglobin concentration g per 100 ml	Total circulating red corpuscle mass ml per animal	Blood volume ml per animal	Total circulating haemoglobin g per animal
36	—	14.7	0.79	2.2	0.323
41	10.0	13.9	0.78	1.9	0.264
40	10.7	15.2	0.80	2.0	0.304
42	13.9	15.4	0.75	1.8	0.277
36	10.0	15.4	1.00	2.8	0.431
38	9.3	13.9	0.99	2.6	0.361
40	10.8	11.9	0.72	1.8	0.214
36	9.8	14.7	—	—	—
39	12.4	15.0	0.98	2.5	0.375
39	9.2	14.7	0.78	2.0	0.294
39	—	13.2	0.78	2.0	0.264
39	10.8	13.2	0.70	1.8	0.237
35	10.8	14.3	0.84	2.4	0.343
39	9.6	13.9	0.74	1.9	0.264
39	9.9	15.0	0.74	1.9	0.285
38	10.6	14.7	0.83	2.2	0.323
36	—	13.9	0.86	2.4	0.334
40	11.9	14.3	0.72	1.8	0.257
41	9.5	13.9	0.82	2.0	0.278
40	11.5	14.7	0.84	2.1	0.309
39	10.1	13.9	0.90	2.3	0.320
40	9.1	13.9	1.00	2.5	0.347
Mean: 39	10.5	14.3	0.83	2.1	0.305
S.E. ± 0.4	± 0.3	± 0.2	± 0.02	± 0.06	± 0.011

^a Body weight = 30 g. ^b Correction factor = 0.88.

The total red corpuscle and haemoglobin mass in the circulation of the cancerous and the control mice were found to be almost identical. Thus, no true anaemia could be observed in our cancerous mice in spite of the shortened life span of their erythrocytes.

Table 3 shows the blood volume, the total circulating red corpuscle mass and the total circulating haemoglobin of cancerous mice studied at different times after tumour transplantation. The haemodilution already occurs at five to ten days after tumour inoculation. The tumour weight after ten days was about 0.1 g. The average tumour weight 20 days after tumour inoculation was 1.5 g. The groups of mice killed at 30 and at 40 days after tumour inoculation had a tumour weight of between 2.5 and 3.0 g. In the last group the possibility of bleeding into the necrotic tumour tissue could not be excluded. No gross ulcerations of the tumour could be observed in the mice investigated.

Radioiron studies

Figure 1 shows the percentage of ⁵⁹Fe present in spleen, liver and whole blood of cancerous and control mice killed at six hours and at four days after intraperitoneal injection of 1 microgram iron as ammonium-citrate labelled with 1 microcurie ⁵⁹Fe.

Table 2. Blood volume, total circulating red corpuscle mass, and total circulating haemoglobin of male TE mice bearing Ehrlich ascites carcinoma growing in the solid form.^a

Body haematocrit ^b %	Number of red corpuscles $\times 10^6$ per mm ³	Haemoglobin concentration g per 100 ml	Total circulating red corpuscle mass ml per animal	Blood volume ml per animal	Total circulating haemoglobin g per animal
37	7.4	12.6	0.85	2.3	0.290
37	9.4	12.9	0.85	2.3	0.296
39	11.0	14.3	0.93	2.4	0.343
41	10.5	15.0	0.94	2.3	0.345
40	9.2	13.6	0.88	2.2	0.299
40	9.7	13.6	0.76	1.9	0.258
41	10.9	14.1	1.02	2.5	0.352
37	8.0	12.4	0.85	2.3	0.285
37	9.5	13.9	0.85	2.3	0.320
40	9.8	13.9	0.84	2.1	0.292
37	9.4	12.9	0.92	2.5	0.322
38	10.9	13.9	1.03	2.7	0.375
39	8.2	13.2	0.89	2.3	0.303
41	9.5	14.3	1.15	2.8	0.400
40	8.3	13.9	1.00	2.5	0.348
35	8.0	13.2	0.73	2.1	0.277
35	8.1	13.2	0.70	2.0	0.264
40	8.8	13.2	0.88	2.2	0.290
35	8.1	12.2	0.98	2.8	0.342
33	7.4	11.0	0.95	2.9	0.319
36	8.9	13.9	—	—	—
33	7.9	12.2	—	—	—
38	8.4	12.6	0.99	2.6	0.328
36	8.5	12.6	0.90	2.5	0.314
36	9.4	13.9	0.93	2.6	0.361
28	5.9	8.4	0.85	3.0	0.255
41	9.7	14.3	0.90	2.2	0.314
37	7.3	12.3	0.85	2.3	0.282
Mean: 37	8.9	13.1	0.90	2.4	0.314
S.E. ± 0.5	± 0.2	± 0.2	± 0.02	± 0.05	± 0.007

^a Body weight = 32 g, average tumour weight = 1.5 g. ^b Correction factor = 0.88.

The mice were studied 20 days after tumour inoculation. Each figure is the average of the values obtained for ten animals.

Six hours after administration of the radioiron, the spleen of the cancerous mice has taken up three times more ^{59}Fe than the spleen of the controls, whereas the liver of the cancerous mice only took up half as much ^{59}Fe as the liver of the controls.

Four days after the injection of radioiron, the spleen of the cancerous mice contained 4–5 times more ^{59}Fe than the spleen of the controls, the total amount of ^{59}Fe present in the spleen being only about one third of the amount present after six hours. The total amount of ^{59}Fe present in the liver after 4 days is 1.5 times higher than the amount present after six hours, the ratio of uptake between cancer and control remaining unchanged.

In these experiments, the incorporation of ^{59}Fe into the whole blood was about 1.7 times higher in the cancerous mice than in the controls.

Table 3. Blood volume, total circulating red corpuscle mass, and total circulating haemoglobin of TE mice bearing Ehrlich ascites carcinoma growing in the solid form at different stages of development.^a

Days after tumour inoculation	Body haematocrit ^{b, c}	Number of red corpuscles ^c × 10 ⁶ per mm ³	Haemoglobin concentration ^c g per 100 ml	Total circulating red corpuscle mass ^c ml per animal	Blood volume ^c ml per animal	Total circulating haemoglobin ^c g per animal
Controls	39	10.5	14.3	0.83	2.1	0.305
5	38	10.3	13.9	0.83	2.2	0.306
10	35	8.9	13.2	0.84	2.4	0.316
20	37	9.0	13.2	0.89	2.4	0.316
30	32	7.6	11.6	0.87	2.7	0.313
40	32	6.9	10.9	0.74	2.3	0.250

^a The average body weight of all animals before tumour inoculation was 30 g.
^b Correction factor = 0.88.
^c Each figure is the average of the values obtained from five animals.

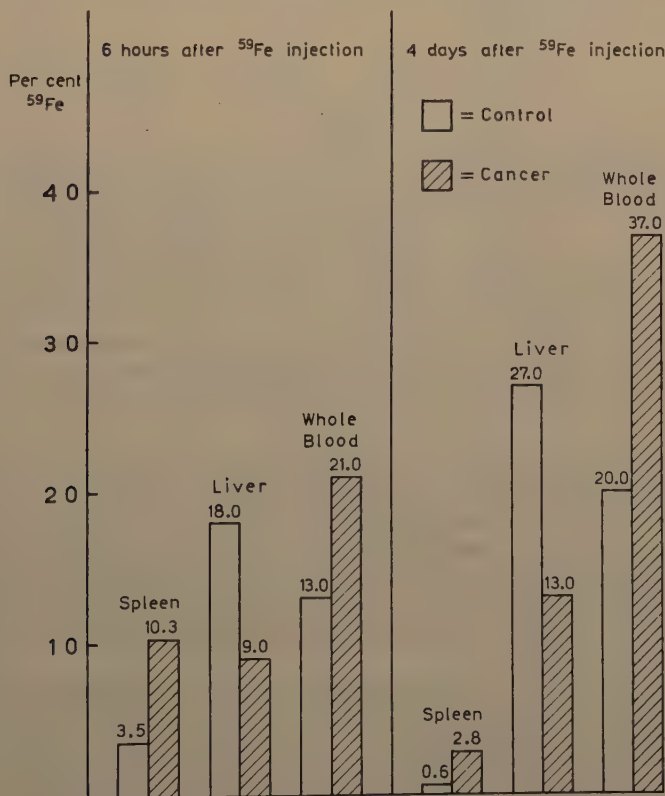


Fig. 1. Percentage of ⁵⁹Fe present in spleen, liver and whole blood of cancerous and control mice at six hours and at four days after intraperitoneal injection of 1 μ C ⁵⁹Fe. Each figure is the average of the values obtained from ten animals.

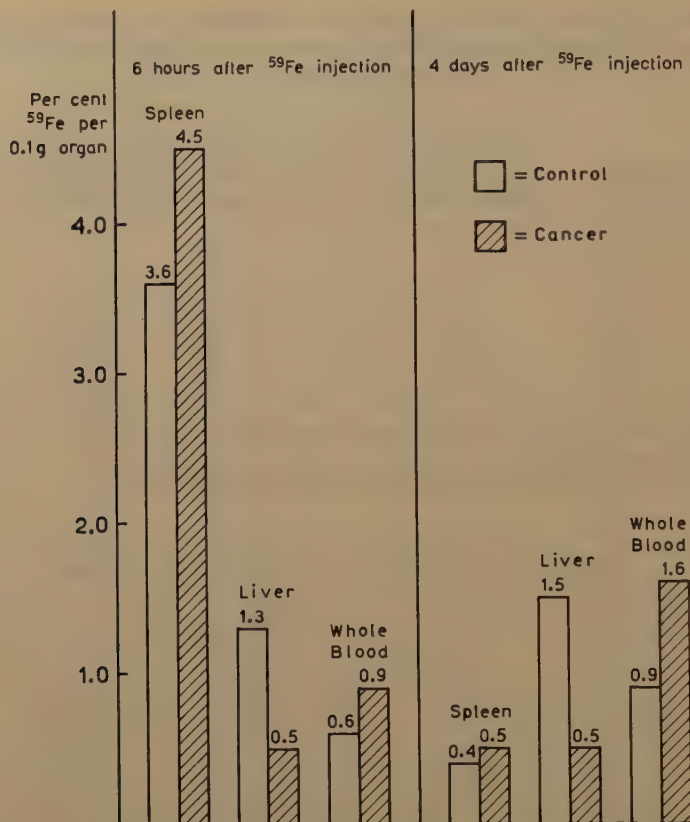


Fig. 2. Percentage of ^{59}Fe taken up by 0.1 g of spleen, liver and whole blood of cancerous and control mice at six hours and at four days after intraperitoneal injection of $1\ \mu\text{C}\ ^{59}\text{Fe}$. Each figure is the average of the values obtained from ten animals.

Figure 2 shows the percentage of ^{59}Fe taken up per unit weight by spleen, liver and whole blood at six hours and at four days after ^{59}Fe administration.

The spleen of both the cancerous and the control mice takes up considerably more ^{59}Fe per unit weight after six hours than the liver and the whole blood. Due to the higher weight of the spleen of the cancerous animals (23), the difference in the ^{59}Fe uptake by the spleen of the cancerous and the control mice is strongly reduced. The figures for the radioiron uptake by the liver and the whole blood are similar to those shown in Figure 1.

The depot iron content of the spleen and the liver of cancerous and control mice were found to be almost equal, the figures being 62 and $61\ \mu\text{g}\ \text{Fe}$ for the spleen and 194 and $193\ \mu\text{g}\ \text{Fe}$ for the liver.

The distribution of the depot iron between the ferritin and the haemosiderin fractions of the spleen and the liver, and their percentage of uptake of radioiron, was also essentially the same in cancerous and in control mice.

Discussion

Blood volume studies

In patients with neoplastic disease a considerable decrease in red blood corpuscle mass and a relative increase in plasma volume was found (6, 7, 9, 10, 12, 13, 24).

In spite of the shortened life span of the erythrocytes of mice bearing Ehrlich acites carcinoma growing in the solid form, the animals developed no true anaemia; the total circulating red corpuscle mass and the total circulating haemoglobin being almost identical with that found in healthy control mice. The increased bone marrow activity thus nullifies the accelerated rate of destruction of the erythrocytes.

The increased erythrocyte production rate in the cancerous animal can also be seen from the enhanced uptake of ^{59}Fe by the circulating erythrocytes, which we found to be almost twice that of the controls.

In contrast to the constant circulating red corpuscle mass, the cancerous animals showed a marked increase of their blood volume, which was due to an increase in plasma volume. This increase of the plasma volume occurred already five days after tumour inoculation and seemed to be proportional to the development of the tumour.

Radioiron studies

Making use of a scintillation counter placed over the spleen Elmlinger *et al.* (25) found that all patients with chronic myelogenous leukaemia studied showed evidence of the production of red corpuscles in the spleen. Some destruction of erythrocytes in the same organ was also indicated (see also 26,27).

The path, which the iron followed from plasma to bone marrow to erythrocytes, was found in most cancer patients studied to be normal (9, 10). The iron content of the liver, spleen and bone marrow was, in most cases, normal or increased (9).

The high uptake of radioiron by the spleen of the cancerous mice, six hours after the administration of the tracer, suggests that the extramedullary erythropoiesis occurring in the spleen of the cancerous mice is appreciably greater than in the spleen of the control mice, where some extramedullary haemopoiesis is normally present. This difference almost disappears, however, if we compare the uptake of radioiron per unit weight. The degree of erythropoiesis in the spleen is very difficult to assess, as the plasma iron turnover and other decisive parameters of the iron metabolism are unknown. Studies are in progress on this problem.

The lower uptake of radioiron by the depot iron fractions of the liver of the cancerous animals is probably due to the fact that the bone marrow and the other erythropoietic tissues of the cancerous mice take up much more iron than those of healthy mice.

The spleen of the cancerous mice also plays an active role in the removal of erythrocytes from the circulation. This is demonstrated by the fact that, four days after administration of the radioiron, the spleen of the cancerous mice contains almost five times more ^{59}Fe than the spleen of the controls.

Applying *in vivo* counting after injection of ^{51}Cr tagged erythrocytes, Ultmann (28) recently came to the conclusion that, in cancer patients with "occult haemolytic anaemia", the spleen participates in the breakdown of red blood corpuscles.

SUMMARY

The circulating red corpuscle mass, the blood volume, the total circulating haemoglobin and the distribution of radioiron in the organs of TE mice bearing Ehrlich ascites carcinoma growing in the solid form have been studied.

The circulating red corpuscles mass was 0.90 ± 0.02 ml and 0.83 ± 0.02 ml, the blood volume 2.4 ± 0.05 ml and 2.1 ± 0.06 ml, the total circulating haemoglobin 0.314 ± 0.007 g and 0.305 ± 0.011 g per animal in cancerous and control mice.

The increase in plasma volume in the cancerous mice occurred 5 days after tumour transplantation and appeared to be proportional to the size of the tumour.

The spleen of the cancerous mice showed a higher erythropoietic activity than the spleen of the control mice.

The spleen of the cancerous mice plays an active role in the removal of erythrocytes from the circulation.

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The geometrical isomers of 2,3-dichlorocinnamic acid

By SVEN LINDENFORS¹

With 4 figures in the text

Of the dichloro-substituted *trans*-cinnamic acids, all except the one with the chlorine atoms in 2,3-position are hitherto known.

trans-cinnamic acids are usually best prepared from the corresponding aldehydes, but as 2,3-dichlorobenzaldehyde is rather laborious to synthesize (1)—a sixstep operation—another way has been tried by the author.

A description was found in the patent literature from 1938 (2) of the reaction between 4-chlorobenzenediazonium chloride and cinnamic acid, leading mainly to 4-chlorostilbene, but also to small quantities of α -4-chlorophenylcinnamic acid. The reaction medium is water and acetone, and sodium acetate is used as a buffer with cupric sulfate as a catalyst.

In five papers (3–7) during the years 1947–51, Kunj Behari Lal Mathur *et al.* describe reactions between benzenediazonium chloride and dicarboxylic acids leading to cinnamic acid derivatives. In (5) and (6) the reactions between a diazonium chloride and acrylic and α -methylacrylic acid are described, giving the corresponding cinnamic acids by β -coupling. The yields are low and a lot of tarry by-products are formed.

The reactions between an aryldiazonium chloride and acrylo-nitrile which lead to α -chlorohydrocinnamitriles have been described by Koelsch (8), and the preparation of β -methyl-2,4-dichlorocinnamic acid from 2,4-dichlorobenzenediazonium chloride and methylcrotonate has been described by Koelsch and Boekelheide (9).

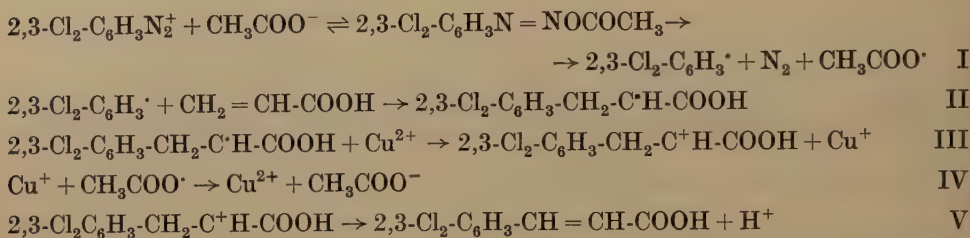
The latest report on the reaction between aryldiazonium chlorides and unsaturated acids is found in the patent literature (10). Thus 2-methoxy-4-nitro-*trans*-cinnamic acid has been synthesized from the corresponding diazonium salt and acrylic acid in a buffered water solution with cupric chloride as a catalyst.

As both 2,3-dichloroaniline and acrylic acid are commercially available at a low price, these chemicals have been found by the author to be the most convenient starting materials for a *one* step synthesis of 2,3-dichloro-*trans*-cinnamic acid.

The coupling of α,β -unsaturated compounds with diazonium salts is an interesting type of reaction of great value in synthetic work. A mechanism has been proposed by Koelsch and Boekelheide (9) which accounts for the products obtained, but surely does not show all the processes involved. For the reaction of

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2,3-dichlorobenzenediazonium chloride with acrylic acid in water buffered with sodium acetate and catalyzed by cupric chloride, the mechanism should be as follows:



In reaction III the cation can also capture a chlorine ion. By the elimination of HCl, which in some cases goes spontaneously, but in other cases must be achieved with alkali, the cinnamic acid is then obtained (8). The present author has boiled the reaction product with diethylaniline to be sure that no chlorohydrocinnamic acid is obtained. The 2,3-dichloro-*trans*-cinnamic acid thus prepared has m.p. 230.5–232.0°.

The *cis*-isomer of 2,3-dichlorocinnamic acid has been prepared from the *trans*-form by irradiation of a solution in 95 % ethanol with ultra-violet rays from a Philips HPK 125 WR burner. The apparatus and general procedure were described by the author in a previous paper (11). From Fig. 1 it is seen that the *trans*-form has a higher absorption than the *cis*-form and that there is a hypsochromic shift for the latter of about 110 Å. The same has been found for other *cis-trans* pairs prepared by the author (11), and for others which has not yet been published. This difference in absorption has its origin in the different geometrical shape of the isomers. In the *trans*-forms the atoms are all in one plane with the possibility of resonance through the whole system but in the *cis*-forms the side chain is out of the plane of the nucleus and the resonance between the aromatic ring and the π -electrons in the double bond is diminished.

In Fig. 2 is shown the connection between radiation time and per cent *cis*-acid generated when 200 ml of a solution of 2,3-dichloro-*trans*-cinnamic acid in 95 % ethanol ($4.23 \times 10^{-3} M$) was irradiated as described for 3,5-dichloro-*trans*-cinnamic acid in a previous paper (11). The isomerization rate is about the same as that for 3,5-dichloro-*trans*-cinnamic acid. The equilibrium concentration (about 93 % *cis*) for the 2,3-isomer is a little higher than for the 3,5-isomer (about 84 % *cis*). The reaction mixture was analyzed at different intervals by taking out 1.00 ml, diluting it to $4.23 \times 10^{-5} M$ and measuring the optical density at 270.0 m μ . From known values of D at different per cent *cis*-acid, the composition has been obtained:

% <i>cis</i>	0	20	40	60	80	100
D ₂₇₀₀	0.725	0.640	0.560	0.482	0.387	0.293

For the preparation of the *cis*-form in larger quantities, a solution with a concentration of $9.21 \times 10^{-3} M$ was irradiated in 250 ml portions for 2.5 hours every time. The *cis*-form was purified by ion-exchange chromatography (see experimental part). The 2,3-dichloro-*cis*-cinnamic acid thus obtained has m.p. 139.5–141.0°.

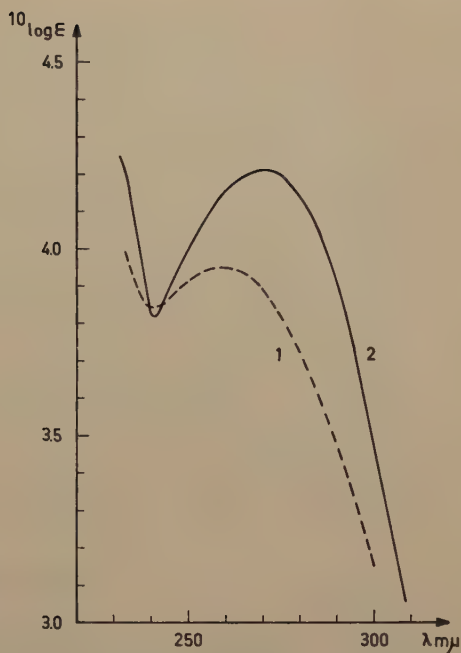


Fig. 1. Ultraviolet absorption of 2,3-dichloro-*cis*-cinnamic acid (broken line) and 2,3-dichloro-*trans*-cinnamic acid in 95 % ethanol (solid line).

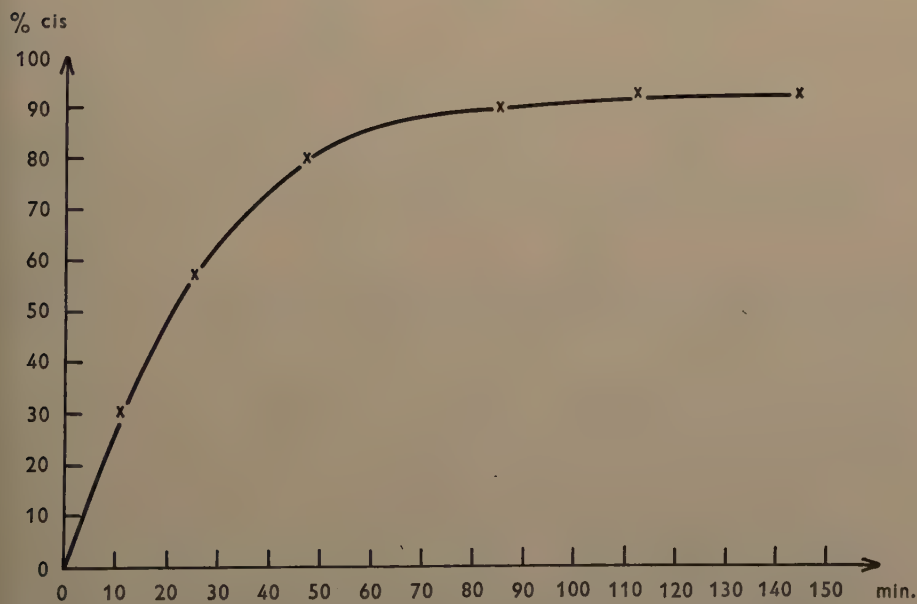


Fig. 2. Isomerization of 2,3-dichloro-*trans*-cinnamic acid in 95 % ethanol.

In Figs. 3 and 4 are given the infrared spectra of the isomers taken in KBr phase. As seen, the peak at about $10.2\ \mu$, which has been considered characteristic of *trans*-forms, is present in both spectra. This was found before by the author in the case of 3-chloro- and 3,5-dichlorocinnamic acid (11). When the spectra are taken in a solvent which has no absorption at $10\ \mu$, the peak from the *cis*-acid disappears, while the *trans*-peak is still there. Carbon disulfide was used previously by the author, but in the case of 2,3-dichlorocinnamic acid the solubility of both isomers is too low to give an absorption from which any conclusions can be drawn. Acetone, which has no absorption about $10\ \mu$, was tried and gave good information, e.g., the *cis*-peak disappears while the *trans*-peak still remains. Another bond which perhaps may be used in the determination of *cis-trans*-structure is the one at $8.00 \pm 0.03\ \mu$, which was found in five *cis*-isomers. It is a broad band and the strongest in the whole spectrum. In the corresponding *trans*-forms this band, which has its origin in the carboxyl group is displaced at least $0.12\ \mu$ to one or the other side of the $8.00\ \mu$ line, and is much weaker and narrower.

The isomers will be investigated for their auxin activities by Professor B. Åberg and the results published in a separate paper.

Experimental

2,3-Dichloro-*trans*-cinnamic acid

24.3 g (0.15 mole) freshly distilled 2,3-dichloroaniline in 350 ml of water and 42 ml concentrated hydrochloric acid was diazotized with 10.8 g solid sodium nitrite at $0-5^{\circ}\text{C}$. The solution was cooled to -5°C , and 15.5 ml acrylic acid (containing 730 g/l, estimated by titration), 7.5 g cupric chloride and 70 g sodium acetate were added, whereupon the temperature fell to about -15°C . The reaction mixture was stirred overnight, with the flask in the freezing-mixture while the temperature was allowed to reach room temperature. The solution was then kept at this temperature for about six hours without stirring. The brown precipitate was then filtered off, boiled with 20 g NaHCO_3 in 750 ml of water, the solution filtered and finally acidified with 6 *N* hydrochloric acid. 18 g of air dried crude product was obtained (55 %). The crude product was boiled for five minutes with five times its own weight of diethylanilin. The product was then recrystallized from 85 % formic acid and from 95 % ethanol, in both cases together with some charcoal. M.p. $230.5-232.0^{\circ}$. The melting points in this paper have been determined according to the microscopical methods of Kofler and Kofler (12) and are thus corrected.

Anal. Subst., 24.38 mg: 4.45 ml of 0.05048 *N* NaOH (combustion according to Grote-Krekeler).

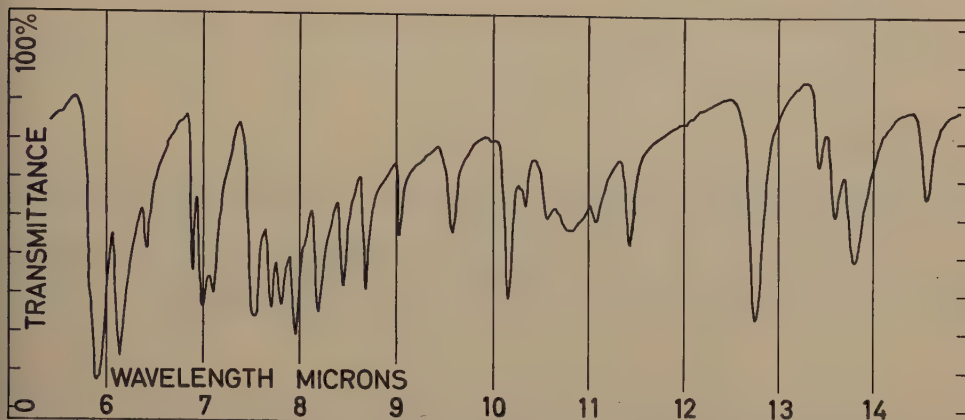
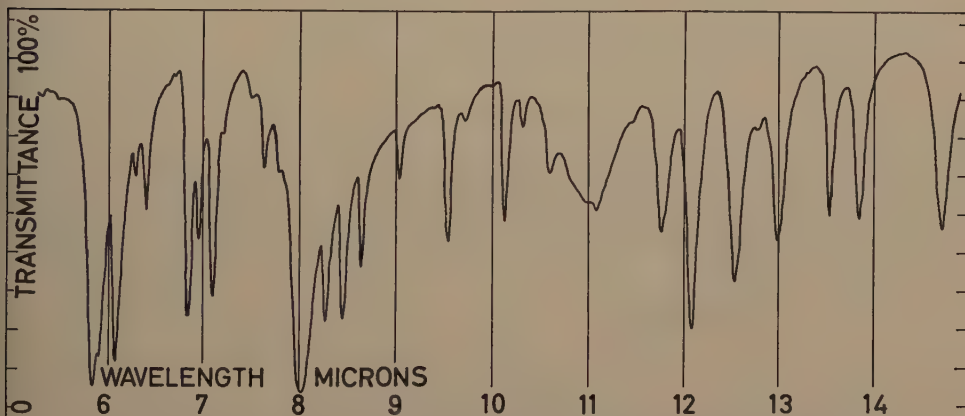
Subst., 221.0 mg: 10.22 ml 0.1001 *N* NaOH.

$\text{C}_9\text{H}_6\text{O}_2\text{Cl}_2$ (217.05): Calc. Cl 32.67; equ. wt. 217.05.

Found Cl 32.73; equ. wt. 216.0.

2,3-Dichloro-*cis*-cinnamic acid

A solution of the *trans*-acid in 95 % ethanol was irradiated with ultra-violet light under circumstances which have been described by the author in a previous

Fig. 3. IR absorption curve of 2,3-dichloro-*trans*-cinnamic acid in KBr phase.Fig. 4. IR absorption curve of 2,3-dichloro-*cis*-cinnamic acid in KBr phase.

paper (11). The concentration was $9.21 \times 10^{-3} M$ and 2×250 ml was irradiated, each portion for 2.5 hours. The ethanol was then driven off, and the solid product extracted with 3×20 ml of cold benzene. 0.75 g crude product was obtained. The *cis*-form was purified by ion-exchange chromatography in a way described by the author in a previous paper (13). 0.65 g crude product was dissolved in 60 ml 75 % ethanol and adsorbed on the IRA-400 portion of a column containing 58×13 mm IRA-400 and 165×13 mm IR-4B. The main part of the *trans*-acid was eluted with 0.005 *N* HCl in 75 % ethanol. When the *cis*-form was then eluted with 0.3 *M* NaCl in 75 % ethanol, the first five ml contained *trans*-acid together with *cis*-acid. About 400 ml HCl solution and 150 ml NaCl solution were used. The ethanol was driven off from the NaCl fractions (except the first five ml), the water solution was acidified with 2 *N* HCl, and the acid filtered off and recrystallized once from petroleum ether (b.p. $40-60^\circ$). M.p. $139.5-141.0^\circ$.

S. LINDENFORS, *Isomers of 2,3-dichlorocinnamic acid*

Anal. Subst., 35.55 mg; 6.50 ml of 0.05048 *N* NaOH (combustion according to Grote-Krekeler).

Subst., 231.6 mg; 10.70 ml 0.1003 *N* NaOH.

$C_9H_6O_2Cl_2$ (217.05) Calc. Cl 32.67; equ. wt. 217.05.

Found Cl 32.67; equ. wt. 215.9.

All measurements in the ultra-violet have been made with a Beckman Model DU Spectrophotometer with calibrated cells.

A Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prisms was used for the infrared absorption measurements. The acids were examined as solid pressed potassium bromide plates and in acetone.

SUMMARY

2,3-Dichloro-*trans*-cinnamic acid has been synthesized by the reaction between 2,3-dichlorobenzenediazonium chloride and acrylic acid in a buffered water solution with cupric chloride as a catalyst.

2,3-Dichloro-*cis*-cinnamic acid has been prepared by the isomerization of the corresponding *trans*-acid in 95 % ethanol with ultra-violet radiation.

The UV- and IR-spectra for the geometrical isomers are given.

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Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, March 1958.

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An improved method of preparing 3.5-dichlorobenzaldehyde

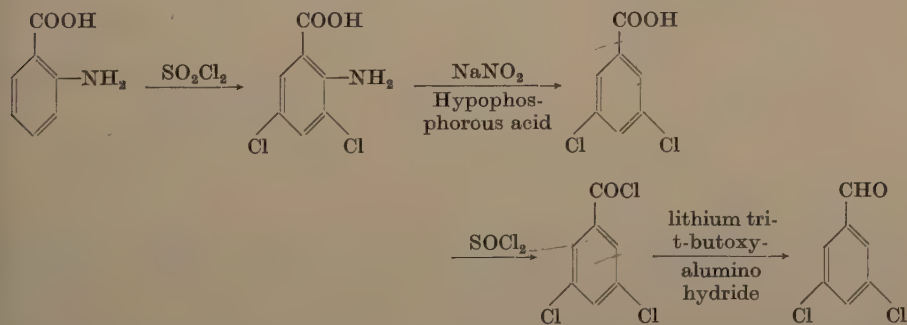
By SVEN LINDENFORS¹

With 1 figure in the text

During an investigation of the geometrical isomers of dichloro-substituted cinnamic acids the problem of synthesizing 3.5-dichlorobenzaldehyde appeared, as this substance leads to the desired *trans*-cinnamic acid by condensation with malonic acid in pyridine.

Asinger and Lock (1) synthesized the aldehyde in a six steps synthesis starting from 2-acettoluidine. The method gives satisfactory results but it has the disadvantage of being very time-consuming and inconvenient because of the great amount of starting material required to get the end product in practical yield.

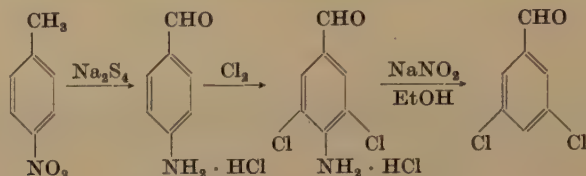
Starting from the acid chloride several ways are open to the corresponding aldehyde, *e.g.* the Rosenmund reduction, the hydrolytic decomposition of Reiser's compounds, the method of Grundmann (via diazo ketone, acetoxy ketone and the corresponding glycol), reductive desulfurization of thiol esters, and reduction with lithium tri-*t*-butoxyaluminumhydride (2). With the acid chlorides the following way seems to be one of the most convenient:



3.5-dichlorobenzaldehyde has not hitherto been synthesized by this method but all intermediates have been prepared by different authors (3) (4) (5). Cohen and Briggs (5) used PCl₅ for preparing the acid chloride but SOCl₂ seems to be better as only gaseous by-products are obtained. 3.5-dichlorobenzoylchloride has not been reduced by the new method of Brown and McFarlin but it is most probable that this could be done as other acid chlorides with electron-attracting groups in the nucleus have been reduced to the aldehyde with great success.

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The most rapid way to introduce the aldehyde group and two chlorine atoms in 1,3,5-position in the benzene nucleus seems to be according to the following scheme:



The interesting oxidation-reduction of 4-nitrotoluene has been systematically investigated by Beard and Hodgson (6). The oxidation of the methyl group to the aldehyde group is brought about by the sodium polysulphide (Na_2S_4). The hydrogen sulphide produced is converted into sodium hydrosulphide by the alkali present. The hydrosulphide together with the sodium sulphide then reduce the nitro group. The method gives about 75 % yield. The aminobenzaldehyde is obtained as an oil which darkens rapidly as the result of condensations involving the two functional groups. This product is boiled under reflux for some minutes with concentrated hydrochloric acid, the solution is cooled and the crystals filtered off. In this form the product can be stored unchanged.

The chlorination of 4-aminobenzaldehyde hydrochloride has been described by van de Bunt (7). It must be carried out carefully, otherwise 2,4,6-trichloro aniline is also obtained. To get the right amount of chlorine the gas is best generated from a known amount of potassium permanganate and concentrated hydrochloric acid. The crude chlorination product can be directly used in the last step.

The diazotisation was carried out in ethanol and the aminogroup eliminated by keeping the solution at 60–65°C for some hours. The aldehyde was steam distilled and finally recrystallized from petroleum ether.

Finally the aldehyde was condensed with malonic acid in pyridine and the corresponding *trans*-cinnamic acid compared with that from the aldehyde synthesized according to Asinger and Lock (1). That the two acids are identical has been shown by infrared spectra (Fig. 1) and mixed melting point.

Experimental

4-Aminobenzaldehyde

The method of Beard and Hodgson (6) was used. As the 4-aminobenzaldehyde hydrochloride is stable the present author has started with 50 g 4-nitrotoluene instead of 10 g. Using the corresponding amounts of the other chemicals and boiling for four hours the yield was 65–70 %. The unstable amino aldehyde was decanted and boiled for about 15 minutes with concentrated hydrochloric acid under reflux. After cooling the hydrochloride was filtered off and air dried. No further purification for the next step is necessary.

4-Amino-3,5-dichlorobenzaldehyde

The crude product above was chlorinated according to van de Bunt (7) in water and glacial acetic acid (1:3) with a determined amount of chlorine, generated from potassium permanganate and concentrated hydrochloric acid. The

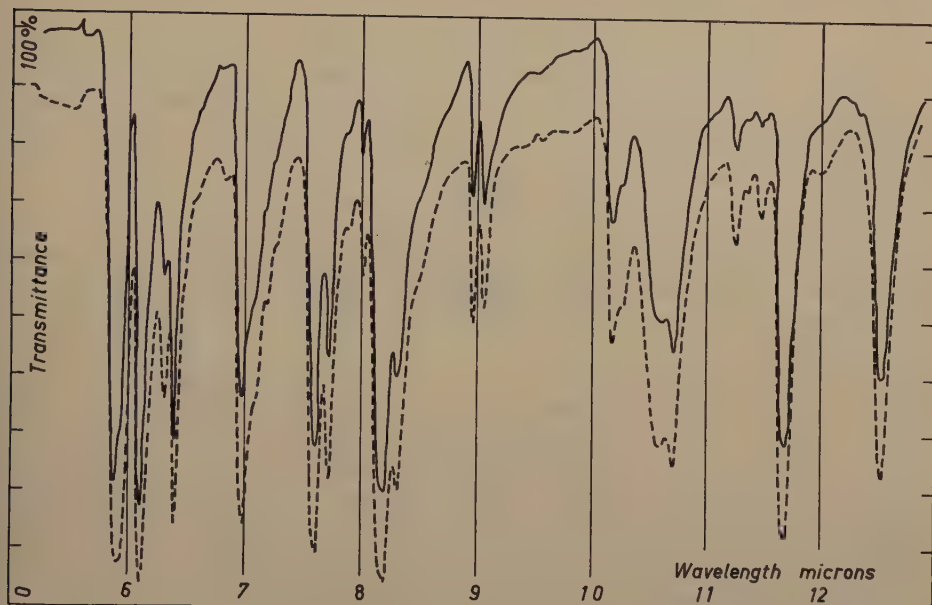


Fig. 1. IR absorption curve of 3,5-dichloro-*trans*-cinnamic acid synthesized from the aldehyde prepared by Asinger and Lock (1) --- and in this investigation —.

present author has found that the results from the chlorination of 500 ml portions is as good as for 250 ml portions, as recommended by van de Bunt, if the solution is thoroughly stirred. The chlorination time for every 500 ml portion was about 40 minutes. After the chlorination the solution is made alkaline with NaOH under cooling and the crude product filtered off and dried *in vacuo*.

3,5-Dichlorobenzaldehyde

25 g of the crude chlorinated aldehyde was poured into 400 ml 95 % ethanol in 45 ml concentrated hydrochloric acid and diazotized in the usual way with 8.9 g solid NaNO_2 . The solution was then kept at 60–65°C over night. 400 ml of water and 20 ml concentrated hydrochloric acid was added and the solution boiled for about 10 minutes, after which the product was steam distilled and the distillate diluted with water. The aldehyde was finally recrystallized from petroleum ether. M.p. 63.5–64.8°. Asinger and Lock give 65°. The over all yield based on 4-nitrotoluene is about 25 %. This aldehyde when melted together with the one synthesized according to Asinger and Lock gave no m.p. depression. 3,5-Dichloro-*trans*-cinnamic acid prepared from the two aldehydes as described by the author in a previous paper gave the same infrared spectra (Fig. 1) (8).

A Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prisms was used for the infrared absorption measurements. The m.p. was determined in a Reichert hot stage microscope and is thus corrected.

SUMMARY

Methods for preparing 3,5-dichlorobenzaldehyde are discussed. A new route for the synthesis of the aldehyde is described. The aldehyde has been identified by converting it to the corresponding *trans*-cinnamic acid, the infrared spectrum of which is given between 5 and 13 μ .

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Professor Arne Fredga for his kind interest in this work and to Dr. Magnus Matell for valuable discussions.

This investigation has been supported by *Jordbrukets Forskningsråd* to which acknowledgement is gratefully made.

Laboratory of Organic Chemistry, Chemical Institute, University of Uppsala, January 1958.

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Tryckt den 9 september 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

Alcoholysis of lysozyme with boron trifluoride

By PEHR EDMAN and LARS JOSEFSSON

With 4 figures in the text

The study of peptide bond cleavage in proteins has in the past almost exclusively been restricted to aqueous media and a considerable literature, excellently reviewed by Leach (1), is available on the mechanism and kinetics of protein hydrolysis. On the other hand alternative media for cleavage have not attracted much attention. In view of the need in protein structural analysis for methods of selective cleavage, we have undertaken a study of the alcoholysis of proteins. The literature contains few references to this problem, and then only in connection with attempts to esterify the carboxyl groups of proteins with alcoholic acids. Our first attempts, using anhydrous methanolic hydrogen chloride, did not seem promising mainly because the proteins were poorly soluble in the medium and therefore unreactive. We then turned to boron trifluoride which has been extensively used as a catalyst in the esterification of carbonic acids (2) and it was found to be a very powerful agent in the alcoholysis of proteins. Also the boron trifluoride addition complexes with methanol and ethanol are good solvents for proteins.

Experimental

Materials and methods

Lysozyme hydrochloride was prepared from egg white according to Alderton and Fevold (3). The preparation was recrystallized three times and then dried over phosphorous pentoxide at 0.01 mm Hg for 24 hours. Total nitrogen, 17.2 %; amino nitrogen 0.75 %.

Anhydrous methanol and ethanol were prepared according to Lund and Bjerrum (4).

Alcohol-boron trifluoride complexes. Boron trifluoride forms two defined complexes with alcohols, the molar ratios of alcohol to boron trifluoride being respectively 2:1 and 1:1. The complexes are liquids at ordinary temperature and the former is volatile whereas the latter is not (2). The complexes were prepared by passing under protection from moisture and ice cooling boron trifluoride gas (Mathieson Co., N.Y., U.S.A.) through the alcohol until the required amount had been taken up as judged by the weight increase.

Total nitrogen was determined by the micro-Kjeldahl procedure.

Amino nitrogen was determined according to v. Slyke with the modifications introduced by Kendrick and Hanke (5).

N-terminal amino acids were determined by the phenyl isothiocyanate procedure (6, 7, 8).

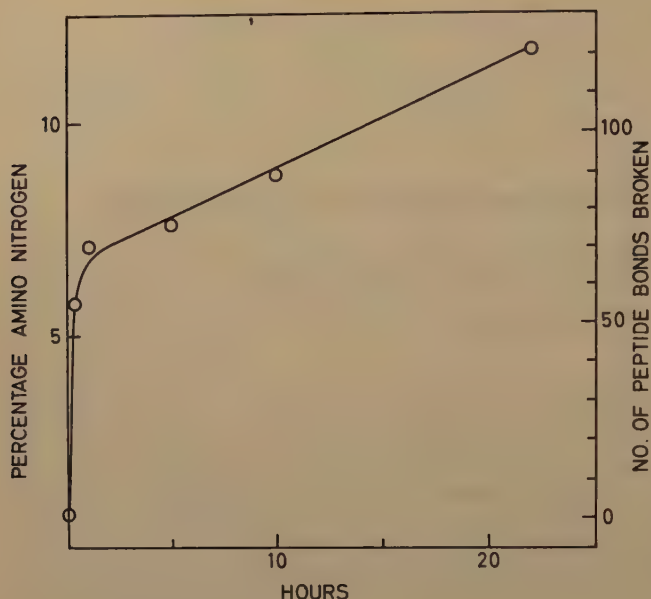


Fig. 1. Methanolysis of lysozyme with boron trifluoride at 100°C.

Results

Methanolysis at 100°C. Eighty mg of dry lysozyme hydrochloride was dissolved in 8 ml of methanol-boron trifluoride (1:1 complex) and the solution refluxed in the boiling water bath with protection against moisture. At suitable intervals 1 ml samples were withdrawn for amino nitrogen and total nitrogen determinations. Prior to these determinations the solvent, which would otherwise have interfered, was removed. To each sample was added an equal volume of methanol and this solution concentrated to dryness *in vacuo* at approx. 80°C. This operation required only a few minutes.

The result of the experiment is presented in Fig. 1. The number of peptide bonds broken per molecule (right hand ordinate in Fig. 1), was calculated on the assumption of 124 peptide bonds per a molecular weight of 15,000 for lysozyme (9,10). The course of the methanolysis is clearly biphasic. A fast initial phase lasting about one hour brings about the cleavage of approximately one half of the total number of peptide bonds present in the protein. This is followed by a much slower phase leading ultimately to the complete cleavage of all peptide linkages.

Methanolysis and ethanolysis at 20°C. A number of 10 mg samples of dry lysozyme hydrochloride was distributed separately in centrifuge tubes. In one series was added to each tube 1 ml of methanol-boron trifluoride (2:1 complex) and in the other series the same volume of ethanol-boron trifluoride (2:1 complex). The tubes were stoppered and kept for varying lengths of time at 20°C. At suitable intervals samples were precipitated by the addition of 10 ml of dry ether and the precipitate centrifuged off. The precipitate was washed once with the same volume of ether and subsequently dried. This procedure ascertained a complete recovery of the protein as confirmed by nitrogen analysis. The sample was then used for amino nitrogen determination or for N-terminal determination. The results of these determinations are summarized in Figs. 2 and 3.

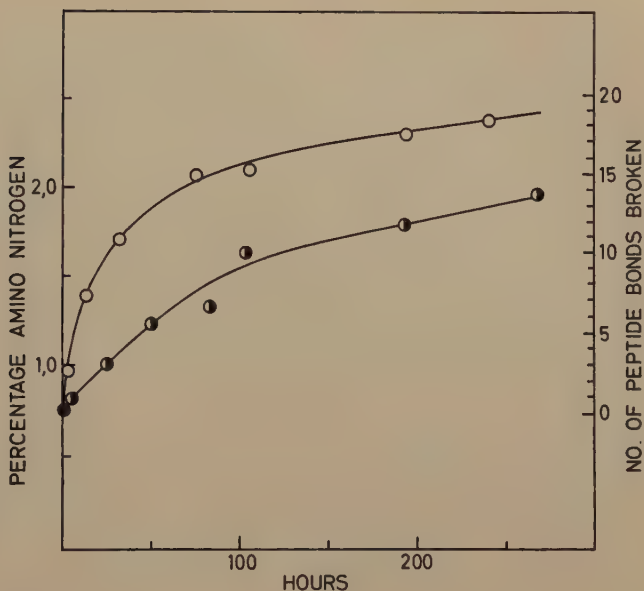


Fig. 2. Methanolysis (—○—○—) and ethanolysis (—●—●—) of lysozyme with boron trifluoride at 20°C.

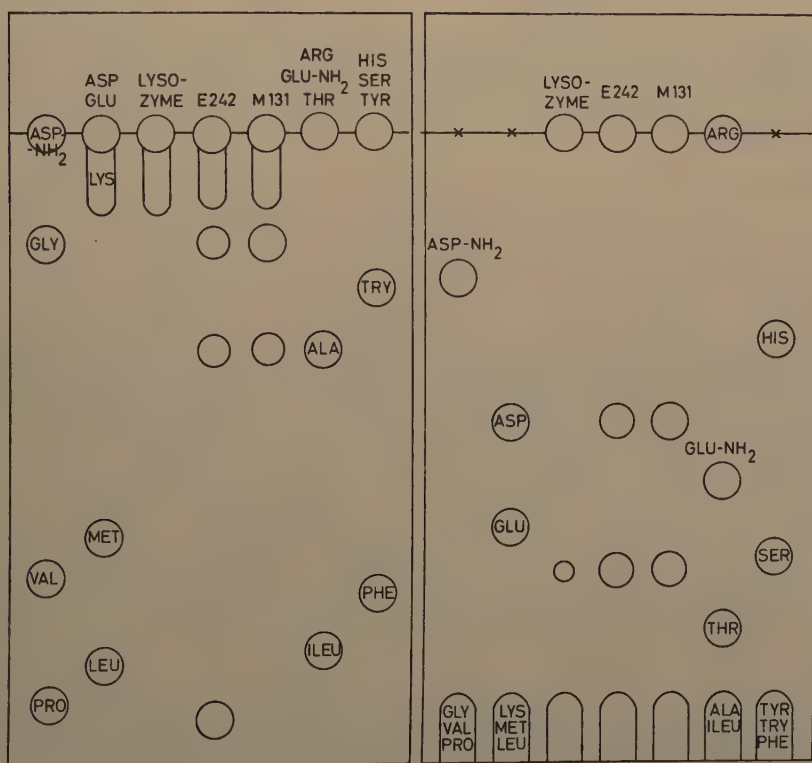


Fig. 3. Paper chromatograms (schematic) of N-terminal determinations during alcoholysis of lysozyme with boron trifluoride at 20°C. M 131 denotes methanolysate after 131 hours, and E 242 ethanolysate after 242 hours. *Left*, solvent system D; *right*, solvent system E. Abbreviations of amino acids are those of Brand and Edsall (18).

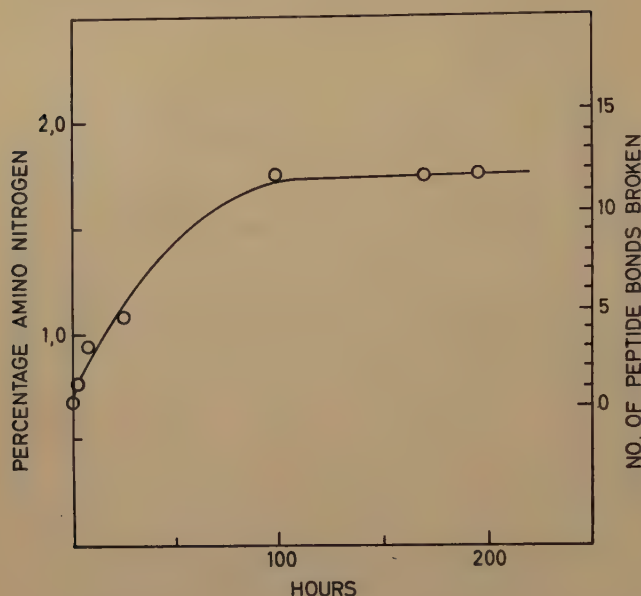


Fig. 4. Methanolysis of lysozyme with boron trifluoride at 11°C.

Methanolysis at 11°C. The experimental procedure was the same as described in the preceding paragraph except that the temperature of the reaction mixture was kept at 11°C. No N-terminal determinations were made. The course of the reaction is presented in Fig. 4.

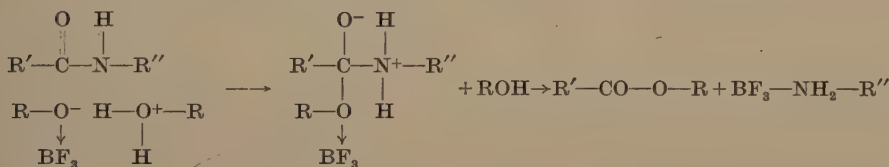
Discussion

This preliminary investigation into the alcoholysis of proteins has demonstrated the potency of boron trifluoride as a catalyst in the breaking of peptide bonds. Methanol-boron trifluoride (1:1) at 100°C brings about a complete cleavage of lysozyme into amino acid methyl esters and the catalytic power of boron trifluoride is thus comparable to that of concentrated aqueous mineral acids. The strikingly biphasic course of the methanolysis has most probably the same explanation as applies to acid hydrolysis, i.e. that dipeptide bonds are more resistant to the attack of positively charged agents owing to the repellent action of the neighbouring ammonium grouping (11).

The results at lower temperature indicate a preferential cleavage of certain peptide bonds. This conclusion is supported by (a) the biphasic course of the reaction and (b) the N-terminal determinations. Extrapolation to zero time (Fig. 2) indicates that in the methanolysis about 15 bonds, and in the ethanolysis about 7 bonds are split considerably faster than the rest. This tendency is even more pronounced in the methanolysis at 11°C, where there is a marked levelling off of the curve (Fig. 4) after the cleavage of 12 bonds. However, this interpretation should be made with some reserve in view of the findings, that N,O-acyl shifts at the hydroxy amino acid residues may easily be induced in anhydrous acids (12, 13, 14, 15). A rearrangement of this kind would of course contribute to an increase in the amino nitrogen, but the magnitude of such a contribution (if any) is difficult to estimate in the present case.

The N-terminal determinations during the early stages of the alcoholysis show a striking predominance of certain amino acids, namely glycine, alanine and aspartic acid.¹ The question then arises if this reflects a certain degree of selectivity on the part of the catalytic agent in splitting those peptide bonds, in which these amino acids enter. It is then relevant that the amino acids cited occur in a high frequency in lysozyme, i.e. glycine 11, alanine 10 and aspartic acid 20 per mole (9, 10) and should therefore be strongly represented in a purely random cleavage. However, the fact that other amino acids which also occur in a high frequency, e.g. 15 leucines, 11 arginine and 10 serine per mole of protein, do not appear until later in the alcoholysis, is not reconcilable with a random cleavage. It therefore seems safe to conclude that the boron trifluoride alcoholysis displays a certain measure of selectivity. It is interesting to compare our results with those obtained in the hydrolysis of lysozyme with 10 *N* HCl at 37°C (16), where it was found that free serine, glycine, alanine, aspartic acid and glutamic acid appeared early in the hydrolysis.

The observed selectivity may not extend to all peptide bonds in which glycine, alanine and aspartic acid enter. It is reasonable to assume, that the steric accessibility of the peptide bond is important for its rate of cleavage, and that therefore also the nature of the carbonylcontributing amino acid of the peptide bond should be of importance in the reaction



because of the bulkiness of the attacking agents. However, this point cannot be definitely settled on the base of the present experimental material.

SUMMARY

Lysozyme has been alcoholized with methanol and ethanol using boron trifluoride as a catalyst. At elevated temperatures lysozyme is completely split into amino acid esters. On the other hand, at low temperature only a few peptide bonds, 7 to 15 in number depending on the nature of the alcohol and the temperature, are split rapidly and the rest at a much slower rate. It has been shown, that this preferential cleavage is restricted to those peptide bonds in which the amino acids glycine, alanine and aspartic acid enter.

This investigation has been supported by a grant from Eli Lilly & Co. to one of us (P.E.) which is gratefully acknowledged.

St. Vincent's School of Medical Research, Melbourne, Australia, and Department of Physiological Chemistry, University of Lund, Sweden.

¹ In the paper chromatograms (Fig. 3) appeared two spots which could not be identified with any of the amino acids known to occur in lysozyme. One of them, only present in the ethanolysate, appeared below proline in solvent system D, and the other between serine and threonine in solvent system E. Lysine was present throughout since it occupies the N-terminal position in the intact lysozyme (17).

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Tryckt den 9 september 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

The heats of hydrolysis of isopropoxy-methyl-phosphoryl fluoride (Sarin) and of the corresponding chloride

By LENNART LARSSON and PER-ERIK BERGNER

With 2 figures in the text

In the course of an investigation of the chemical reactivity of a group of organic phosphorus compounds it was of interest to determine the heat of the alkaline hydrolysis of isopropoxy-methyl-phosphoryl fluoride (Sarin) and of the corresponding chloride. In the field of the thermochemistry of organic phosphorus compounds there is not much work reported. Thompson (1) has determined the heat of combustion of the disodium salt of methyl-dihydroxy-phosphine oxide and Neale and Williams (2, 3) have estimated the average bond energies of a number of phosphorus bonds from reaction calorimetry. The fact that several organic phosphorus compounds are extremely toxic, makes it desirable to work with small samples, thus giving rise to difficulties in doing such measurements without too great a loss of accuracy. The calorimeter used in the present study was of the simple Dewar-vessel type and was a modification of that described by Pritchard and Skinner (4).

Experimental

Calorimeter

A silvered Dewar flask, provided with a nylon stopper around which a rubber ring was stretched, served as the calorimeter vessel (Fig. 1). A mica sheet divided the reaction vessel into two parts; one part contained a thermistor, sealed in a thin glass tube, and the other part contained a mechanism for breaking the glass ampoules, a stirrer, and a heating coil for the calibration.

The thermistor (Type T-18-27, Hafaprodukter, Stockholm) was connected to an ordinary Wheatstone bridge (Fig. 2). From experiments it was found that a change in temperature of 0.1°C could be read off with a standard deviation of about 0.1 %.

The mechanism for the breaking of the glass ampoule was made of stainless steel. The ampoule rested on a jagged support and was held in place by gentle pressure from a plunger. The ampoule was broken by crushing it against the support through screwing down the plunger. The stirrer was an ordinary glass propeller (diameter 1.7 cm) rotating with 200 r.p.m., and its glass axis passed through a teflon bearing in the nylon stopper.

The heating coil consisted of a 10 cm long manganin wire, with a diameter

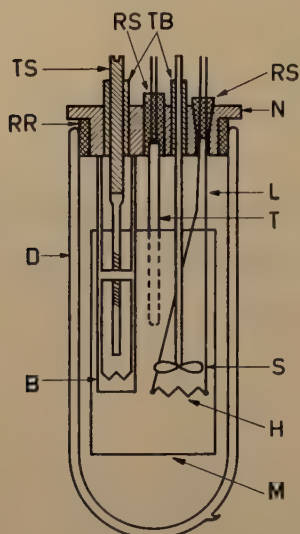


Fig. 1.

B = Ampoule breaker
D = Dewar vessel
H = Heating coil
L = Copper leads
M = Mica sheet
N = Nylon stopper

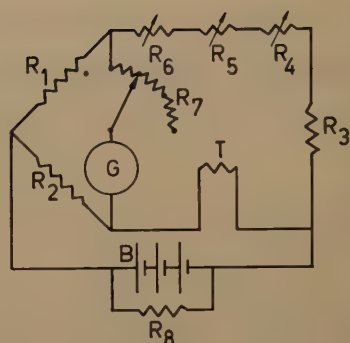


Fig. 2.

Fig. 1. Calorimeter vessel.

RR = Rubber ring
RS = Rubber stopper
S = Stirrer
T = Thermistor
TB = Teflon bearing
TS = Teflon shaft

Fig. 2. Thermistor circuit.

B = 4.5 volt dry cell
G = Light spot galvanometer; sensitivity = 0.4×10^{-9} amp./unit; inner resistance = 9000 ohms
T = Thermistor; 20 kohms at 25°C; $\Delta R/\Delta T \approx 800$ ohms/°C
 $R_1 = R_2 = 50$ kohms, wire-wound

$R_3 = 15$ kohms, wire-wound
 $R_4 = 0.1$ kohms, wire-wound potentiometer
 $R_5 = 1$ kohm, wire-wound potentiometer
 $R_6 = 10$ kohms, wire-wound potentiometer
 $R_7 = 8 + 12 + 36$ kohms, wire-wound
 $R_8 = 3900$ ohms, carbon resistor

of 0.1 mm, and its ends were soldered to two copper leads, which passed through the nylon stopper. Before being mounted on the mica sheet, the coil was dipped into an epoxy varnish (Virapox 5, Syntem Trådlack A.B., Stockholm) and dried at 180°C for four hours. This procedure was repeated four times. The coating obtained in this way was resistant to alkali and prevented electrolysis, but was thin enough to allow good heat exchange with the surrounding liquid. The resistance of the coil was found to be 6.195 ohms at 25°C, by means of a Siemens' precision bridge with an accuracy of 0.02 %. A 6-volt lead accumulator was used as a source of calibration energy and the current through the heating coil was measured by a Siemens' ammeter with an accuracy of 0.2 %. The time of the electrical calibration was controlled by a micro-switch connected between the coil and the accumulator and regulated by a camshaft wheel mounted on a synchronous motor. The time of the energy input was found to be 45.47 seconds with

an error of less than 0.05 seconds. The current through the heating coil could be varied by a precision potentiometer.

The Dewar vessel, the Wheatstone bridge and the dry cells were placed inside an air thermostat, while the other parts of the calorimeter equipment, together with a light-spot galvanometer, were placed outside. The temperature of the thermostat was $25.0 \pm 0.05^\circ\text{C}$, and the temperature inside the calorimeter vessel never deviated more than 0.4°C from that of the thermostat.

Procedure

The ampoule, containing the sample, was placed in the ampoule breaker and 225 ml of 0.2 *M* sodium hydroxide or distilled water, thermostated overnight, was poured into the calorimeter. After about one hour, when equilibrium was reached, the reaction was started by breaking the ampoule. The deflection of the galvanometer was noted from 5 minutes before to 13 minutes after the start of the reaction. The adiabatic change of temperature was determined from a time-temperature curve by a conventional method (5). At each experiment two calibrations were performed, one before and one after the ampoule was broken. In order to give the calibrations equal weights the heat of the reaction had to be very near the centre of the interval defined by the two calibration energies, and to minimize the standard deviation this interval had to be as narrow as possible.

In no experiments did the increase of temperature, gained from the heat of the reaction, exceed 0.15°C . The small change of temperature has the advantage that the cooling curves before and after the reaction will be parallel, which increases the accuracy of the graphical determination of the change of temperature. The standard deviations, given for the mean values in the results, are purely calorimetric errors, calculated from the knowledge of the standard deviation of the different measurements as observed and of the graphical procedure.

Materials

The Sarin (6) and isopropoxy-methyl-phosphoryl chloride (7) used were synthesized in this institute and freshly distilled before use. Isopropoxy-methyl-hydroxyphosphine oxide was prepared from the corresponding pyrophosphate, bis-(isopropoxy-methyl-phosphoryl) oxide, according to the following method:

1.122 g of the pyrophosphate was dissolved in 25 ml of 0.5 *M* sodium hydroxide. By titration the hydrolysis was found to be completed after 10 minutes, and the solution was poured through a proton saturated ion exchanger (Dowex 50 250/500), which was washed with 50 ml of distilled water. The volume was reduced under vacuum to 7 ml. During this procedure the temperature never exceeded 35°C . The concentration of the acid was now found to be 1.116 mmoles/g.

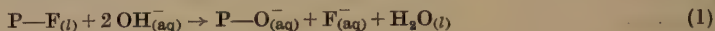
Bis-(isopropoxy-methyl-phosphoryl) oxide was synthesized from isopropoxy-methyl-phosphoryl chloride analogously to tetraethyl pyrophosphate (8). B.p. $85^\circ/0.1\text{ mm Hg}$; $n_D^{25} = 1.4305$; $d_4^{25} = 1.121$.

Found: C, 37.2; H, 7.83. Calc. for $\text{C}_8\text{H}_{20}\text{O}_5\text{P}_2$ (258.2): C, 37.2; H, 7.81.

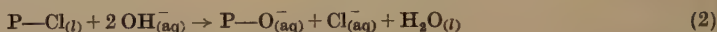
Results

The hydrolysis of Sarin and of isopropoxy-methyl-phosphoryl chloride in 0.2 *M* sodium hydroxide can be considered to reach completion instantaneously, which

minimizes the calorimetric errors. The heat of hydrolysis of these two compounds is given below, where the former compound is represented by P-F, the latter by P-Cl, and P-OH denotes the product of hydrolysis, *isopropoxy-methyl-hydroxyphosphine oxide*. All the data given below refer to 25°C.



Weight (g)	0.0858	0.1001	0.1096	
ΔH_1 (kcal. mole ⁻¹)	-46.8	-47.2	-46.5	Mean -46.8 ± 0.2



Weight (g)	0.0760	0.0582	0.0728	
ΔH_2 (kcal. mole ⁻¹)	-55.8	-54.7	-54.1	Mean -54.9 ± 0.3

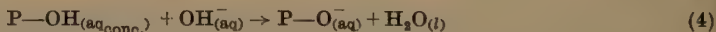
The deviations in the values in the latter reaction are greater than expected which may probably be due to *isopropoxy-methyl-phosphoryl chloride* being easily hydrolyzed by the moisture of the atmosphere, and no rigorous measures being taken to protect the compound during its handling.

The determination of the heat of solution of Sarin in water will not be affected by the heat of its hydrolysis because the hydrolysis in water can be neglected during the time in question. This prediction is supported by the fact that the cooling curves before and after the reaction are parallel.



Weight (g)	0.6612	0.6541	
ΔH_3 (kcal. mole ⁻¹)	-3.0	-3.2	Mean -3.1 ± 0.04

The determination of the heat of neutralization of the acid formed in the hydrolysis and its heat of solution gave the following results:

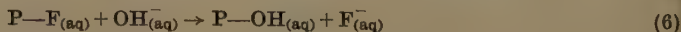


Weight (g)	0.7658	0.7284	0.7474	
ΔH_4 (kcal. mole ⁻¹)	-17.9	-17.8	-17.5	Mean -17.7 ± 0.1

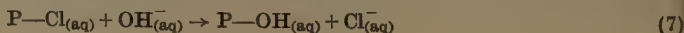


Weight (g)	0.7266	0.7268	
ΔH_5 (kcal. mole ⁻¹)	-3.6	-2.5	Mean -3.1 ± 0.2

From the data given above the heat of the alkaline hydrolysis of Sarin



at infinite dilution at 25°C can be calculated according to $\Delta H_6 = (\Delta H_1 - \Delta H_3) - (\Delta H_4 - \Delta H_5)$ to be -29.1 kcal. mole⁻¹. Because the heat of dilution of 0.2 M sodium hydroxide is less than 0.01 kcal. mole⁻¹ according to Richards and Rowe (9), it can be neglected. On the presumption that the heat of solution of *isopropoxy-methyl-phosphoryl chloride* is the same as that of Sarin the heat of the reaction



can be calculated analogously to that of Sarin as $\Delta H_7 = -37.2$ kcal. mole⁻¹. Neale and Williams (2) have found that the heat of hydrolysis of the chloride compound in water is -29.5 kcal. mole⁻¹. If their system is transformed to reaction (7) by correcting for the heat of solution of the compound (ΔH_3) and for the heat of neutralization of hydrochloric acid ($\Delta H = -13.3$ kcal. mole⁻¹ at infinite dilution (10)), one gets $\Delta H_7 = -39.7$ kcal. mole⁻¹.

The relation between the heats and rates of the alkaline hydrolysis of Sarin and isopropoxy-methyl-phosphoryl chloride will be discussed elsewhere.

SUMMARY

The heats of the alkaline hydrolysis of isopropoxy-methyl-phosphoryl fluoride and of the corresponding chloride have been determined, and a calorimeter suitable for small amounts of samples has been described. The purely calorimetric errors were estimated to be less than 0.5 %, when the change of temperature was 0.1°C.

ACKNOWLEDGEMENTS

Our sincere thanks are due to Professor G. Ljunggren, Director of this Institute, for the kind interest he has shown in this work.

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Tryckt den 9 september 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

On the biological methylation of histamine

By KERSTIN M. LINDAHL

With 1 figure in the text

In many animals intravenously injected histamine is methylated, and oxidatively deaminated prior to excretion in the urine. Thus 1-methylimidazol-4-acetic acid and also, to a minor extent, 1-methyl-4(β -aminoethyl)imidazole, referred to hereafter as methylhistamine, has been shown to appear in the urine (for references see Schayer 1956 and Lindell 1958). *In vitro* the methylating and oxidative enzyme system has been demonstrated in homogenized cat liver (Kobayashi and Schayer 1956) and in mouse liver slices (Lindahl 1958). To my knowledge it has not been possible to separate the methylating enzyme. However, it might belong to the same group of enzymes as the nicotinamide and guanidino acetic acid methylkinases (Ericson, Williams, and Elvehjem 1955) of which the former has been isolated from e.g. rat liver (Cantoni 1951).

By use of similar methods it has been possible to make a crude enzyme preparation which initiates the methylation of histamine. Mouse liver was ground in sodium acetate buffer (I). Part of the homogenate was centrifuged at 25,000 *g* in the cold, an aliquot of the supernatant liquid (II) was heated to 47°C for 10 min., and centrifuged again at 1600 *g*. Aliquots of the supernatant (III) as well as of I and II were tested for activity in the following manner. The enzyme preparation was incubated with an equal volume of a solution containing C¹⁴-labelled histamine dihydrochlorid (4.6 μ g/ml; 25.77 μ C/mg), methionine, magnesium chloride, adenosine di- and triphosphate, inorganic phosphate, and α -ketoglutarate in tris buffer, pH 7.4. Samples were withdrawn at intervals, and deproteinized, the radioactive reaction products being purified on Dowex-1 and separated by one-dimensional paper chromatography (for details see Lindahl 1958). It was found that in I almost all radioactivity was localized to one peak, with the same *R_f* value as histamine, even in samples withdrawn after 2 hours' incubation (Fig. 1). In II a second peak appeared with an *R_f* value identical with that of methylhistamine. All the radioactivity in III was after 2 hours' incubation localized to the same level as methylhistamine, which implies the formation of roughly 7 μ g methylhistamine/g liver/hr. Thus the activity of the histamine methylating enzyme in the liver preparation increases markedly after high speed centrifugation and heat treatment. There is evidently an increase not only in specific but also in total enzyme activity, which might possibly be due to a gradual removal of inhibitors. In Cantoni's preparations this phenomenon does not occur, which suggests that the histamine methylating enzyme is distinguished from his enzyme. Furthermore, the occurrence of nicotinamide methylkinase differs from what would be

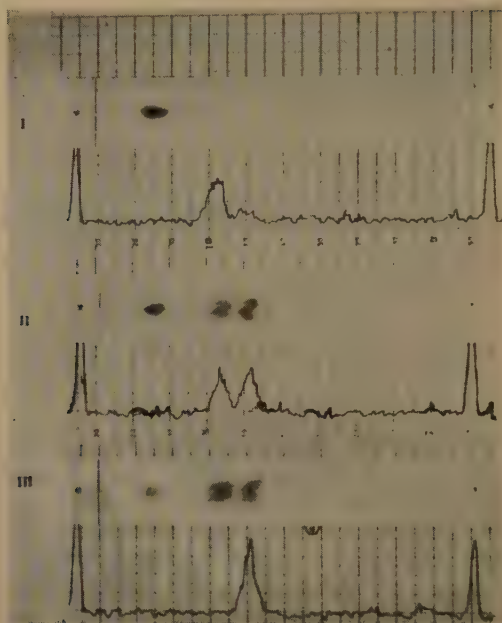


Fig. 1. Paper chromatograms on incubates of C^{14} histamine with mouse liver: I, homogenate; II, supernatant; III, heat-treated supernatant (incubation time 2 hours, see text). Developed in formic acid, 25 % pyridine and tertiary butanol 15:15:70. Assayed for radioactivity in an automatic scanning device FH 452, radioactive guide marks being placed 1 cm above the starting line and below the front line. I: no marker added. II and III: nonisotopic histamine and methylhistamine added. The markers are made visible by ninhydrin.

expected for a histamine methylating enzyme. No methylimidazoleacetic acid was detected by the method used. The occurrence as well as the properties of the histamine methylating enzyme are under investigation.

From the Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden.

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Free zone electrophoresis

Preliminary note

By STELLAN HJERTÉN

With 3 figures in the text

In order to electrophoretically split up a sample containing components of different mobilities into completely separate zones, it is necessary to reduce the convection, resulting from the fact that the density of a zone is different from that of the surrounding solution. A zone electrophoresis is therefore usually conducted in either a stabilizing medium e.g. cellulose powder or in a density gradient. Both these methods often give very good results, but for some purposes they present certain disadvantages. Therefore another method appeared desirable, which would permit zone electrophoretic separation of substances in a free solution medium of homogeneous and constant composition. The stabilization of the zones against the influence of gravity was arrived at by performing the electrophoresis in a horizontal, *rotating* tube. The speed of the rotation was 15–60 r.p.m. The tubes originally used were made of glass, which however caused a strong electroosmotic flow. This troublesome effect has been strongly reduced by making the tube from perspex. As an increase in viscosity of the buffer solution brings about a decrease in osmosis and also a better stabilization of the zones, the electrophoresis can as an alternative be performed with advantage in a buffer containing 0.5 % methylcellulose. The experiments shown in this paper are,

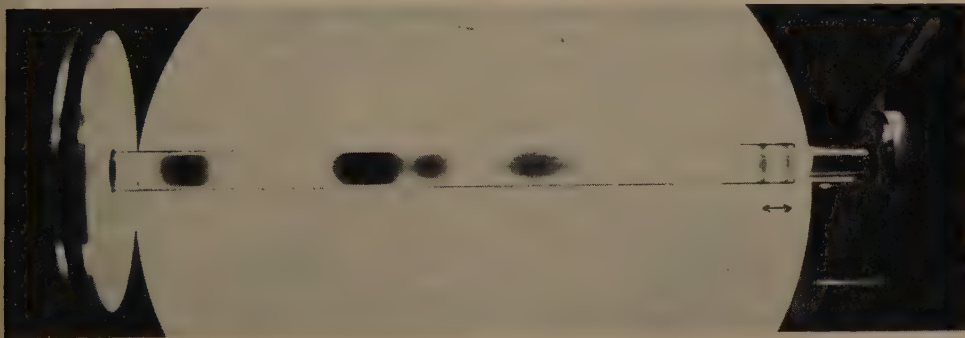


Fig. 1. Free zone electrophoresis in 0.03 *M* sodium borate buffer, pH 8.9, of a sample containing four dye stuffs, naphthol green, phenol red, bromo thymol blue and methyl orange. The time for the run was about 2 hours. The current was adjusted to 2 mA. The width of the starting zone is indicated by an arrow (↔). The inner diameter of the electrophoresis tube was 0.6 cm.

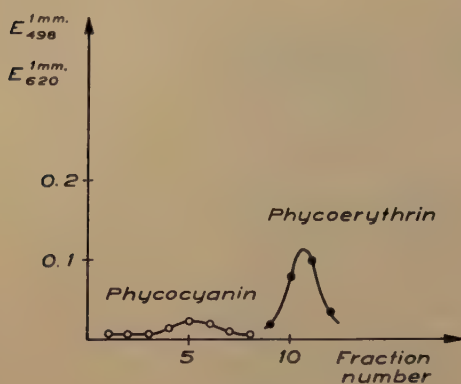


Fig. 2.

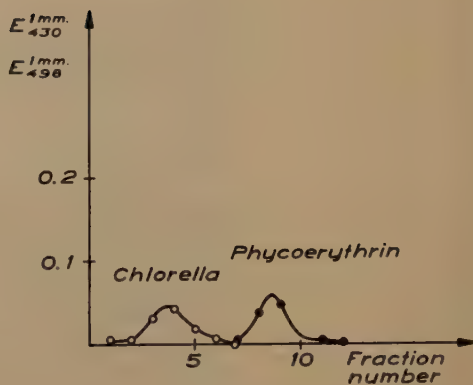


Fig. 3.

Fig. 2. Free zone electrophoresis of a mixture of phycoerythrin and phycocyanin. The run was conducted in 0.04 *M* sodium phosphate buffer, pH 6.8, at 5 mA for 3 hours. With the aid of a polyethylene tubing, fractions were taken out for absorption measurements at 498 $m\mu$ (—●—●—) and at 620 $m\mu$ (—○—○—) in a 1-mm cell. The inner diameter of the electrophoresis tube was 0.5 cm.

Fig. 3. Free zone electrophoresis in 0.05 *M* sodium phosphate buffer, pH 6.8, of a mixture of chlorella and phycoerythrin. The current was adjusted to 5 mA. The time required for the separation was 4 hours. Absorption measurements of the different fractions, taken out with the aid of a polyethylene tubing, were made in a 1-mm cell at 430 $m\mu$ (—○—○—) and at 498 $m\mu$ (—●—●—). The inner diameter of the electrophoresis tube was 0.5 cm.

however, carried out in buffers without any methylcellulose. To reduce the disturbing effect of the osmotic flow to a minimum, a hydrodynamic counter flow has also been used in some of the experiments.

By these procedures free zone electrophoresis separations of low-molecular-weight substances (DNP-amino acids; dye-stuffs, Fig. 1) as well as of proteins (Fig. 2) have been carried out. Probably also particles and cells can be electrophoretically investigated in this way (Fig. 3).

Studies of the method are in progress and in a future publication a more detailed description will be given.

I am indebted to Prof. A. Tiselius for his kind interest in this work.

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Studies on yeast invertase

IV. Action of impurities in the enzyme preparations on the inhibition of the enzyme by silver ions

By KARL MYRBÄCK

With 9 figures in the text

All yeast invertase preparations are strongly inhibited by silver ions. The degree of inhibition caused by a certain concentration of Ag^+ is highly dependent on the pH of the assay mixture (1) and on the nature and concentration of the buffer used. Sodium acetate-acetic acid buffer of low concentration, e.g. 0.01 *N* sodium acetate in the assay mixture, may be used (2), since the complexing of Ag^+ by the acetate ion in such solutions is so weak that the error introduced is negligible.

If the inhibition by Ag^+ of various invertase preparations is determined at the same pH, buffer concentration and other conditions it is found, as could be foreseen, that the Ag^+ concentration giving a certain inhibition, i.e. the Ag^+ concentration corresponding to a certain relative activity (RA) of the enzyme, is not independent of the degree of purity of the invertase solutions. Among the various impurities in crude yeast autolysates silver-binding substances will almost certainly be present which will more or less protect the invertase against the inhibitor.

The inhibition of yeast invertase by Ag^+ is, within reasonable limits, independent of incubation of enzyme with the inhibitor; the reaction of the enzyme protein with the silver ion is instantaneous and, even in the case of highly purified enzyme, fully reversible. The inhibition is non-competitive, i.e. the substrate concentration does not influence the equilibria enzyme- Ag^+ -impurities.

On the whole it can be said that the extent of inhibition of invertase by a certain Ag^+ concentration increases with increasing purity of the enzyme preparation. However, in many cases it has been observed that, at a fixed Ag^+ concentration, rather crude enzyme solutions are inhibited nearly as extensively as are preparations of the highest purity obtainable. On the other hand partly purified invertase solutions may be comparatively insensitive to Ag^+ . This is of course easy to explain; the nature of the impurities in the enzyme solutions may well be more important than their total amount. The type of yeast, the method for liberation of invertase from the insoluble cell structures, and the purification procedures may all be more or less decisive for the behaviour of a yeast invertase preparation in the presence of silver ions or other inhibitors.

It should be emphasized that a comparison of different yeast invertase (or other enzyme) preparations with regard to their inhibition by Ag^+ cannot be based upon experiments with *one* Ag^+ concentration; the *form* of the inhibition curve, i.e. the

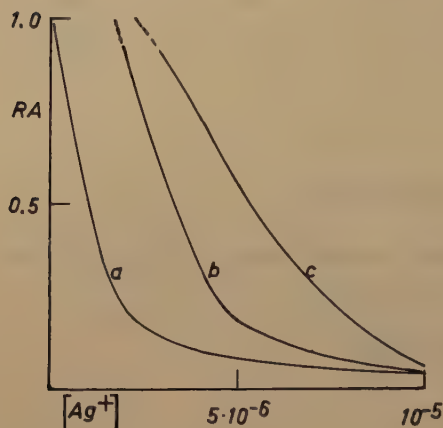


Fig. 1. Inhibition curves at pH = 5.3: *a*, Enzyme B200; *b*, Enzyme A7; *c*, Enzyme A10.

curve obtained by plotting RA versus $[Ag^+]$, may vary strongly. Three examples are given in Fig. 1 (pH = 5.3). Curve *a* shows the inhibition of a highly purified enzyme, described in the following as Enzyme B200. Inhibition is observed at all Ag^+ concentrations, even the lowest. This shows that no measurable amounts of impurities are present in this preparation which bind Ag^+ with a considerably higher affinity than does the enzyme itself. In contrast it is found with the other two invertase preparations (curves *b* and *c*) that the lowest Ag^+ concentrations have no inhibiting action at all on the enzyme, showing that these enzyme preparations contain impurities that bind Ag^+ much more strongly than the enzyme; such impurities could be, e.g., proteins with available HS-groups. Further, the slope of the three curves in Fig. 1 is not the same: curve *c* differs considerably from the other two curves in this respect. If the enzyme were completely free from all impurities with an affinity for Ag^+ , the slope of the inhibition curve would be determined by the dissociation constant of the enzyme- Ag complex alone. However, if the enzyme preparation contains Ag -binding impurities whose silver complexes have dissociation constants of the same order of magnitude as that of the enzyme- Ag complex, the slope of the curve will be changed. Thus it may be concluded, that Enzyme A10 (curve *c*) contains, not only small amounts of some impurity, binding silver more strongly than the enzyme, but also certain amounts of impurities, binding silver with affinities comparable to that of invertase. Impurities of this kind may be present also in Enzyme A7 (Fig. 1, curve *b*) but obviously in smaller amounts. If such impurities are present in the highly purified Enzyme B200 cannot be said with certainty, since yeast invertase has not been obtained in pure condition.

During our studies on the inhibition of yeast invertase observations were made which seem to allow conclusions on the nature of certain contaminating substances in the enzyme solutions and their complex formations with silver ions. These observations may be of value for the understanding of the reaction between groups of the invertase protein and Ag^+ .

Experimental

Activity determination

All experiments were carried out in sodium acetate-acetic acid buffer, usually at pH 5.3. pH was determined in the assay mixtures using the glass electrode. The concentration of sodium acetate was 0.01 *N*. Composition of assay mixture:

4.75 g saccharose,
1 ml acetate buffer (*N* sodium acetate + acetic acid),
Silver nitrate solution and other additions,
Enzyme solution,
Water to 100 ml.

All ingredients except the enzyme solution were mixed and brought to 30°C in a water thermostat. The enzyme solution, usually 1 ml, was added; the reaction rate was determined polarimetrically as described earlier (2, 3); generally 5 samples were measured in each experiment: 10 ml sample + 10 ml *N* sodium carbonate solution, 2 dm tubes. The decrease of rotation is proportional to the reaction time for at least 30 % of the hydrolysis reaction. The decrease of rotation during the first 50 min has been used in the following as an arbitrary measure of the initial reaction rate, i.e. the activity of the amount of enzyme used in the experiment. The relative activity, RA, refers to the activity of the enzyme under the same experimental conditions but without inhibitor; this activity is put = 1.

The dry weight of the enzyme solutions was determined by drying at 100° for 20 h. From reaction rate and dry weight the specific activity of the enzyme preparations was calculated; the *I*f value (v. Euler and Svanberg (4)) was used to express the degree of purity. Since the reaction rate was determined at 30° the corresponding *I*f values are given as *I*f₃₀. Experiments with two enzyme preparations (B15 and B200) are quoted from earlier investigations. In these cases the *I*f activity had been determined at 18° and the *I*f values are *I*f₁₈.

Enzyme preparations

A. Preparations from baker's yeast

Suspension 1. 500 g baker's yeast (dry weight 25 %) was mixed thoroughly with 50 ml ethyl acetate and left at room temperature. After two days the mixture was poured into a large volume of water and centrifuged. The solids were washed in the centrifuge with 5 × 3 liters of water and were then suspended in 500 ml of water. Only about 5 % of the invertase of the yeast was liberated during autolysis and washing. The suspension was stable for months in the refrigerator, and only traces of invertase were liberated during storage, obviously because the proteolytic enzymes, capable of liberating invertase, had been destroyed or removed by the previous treatment. The activity of the suspension was 3.80/ml and the dry weight 143 mg/ml; *I*f₃₀ = 0.32. For the determination of the *A*g inhibition experiments were carried out with 1.0 or 0.6 ml suspension respectively.

Enzyme A1. Autolysis of baker's yeast with 10 % toluene. The filtrate was precipitated with an equal volume of ethanol. The precipitate was dissolved in water and dialyzed. 2 ml samples were used for the determinations. Activity 2.40/2 ml; dry weight 21.6 mg/2 ml; *I*f₃₀ = 1.3.

Enzyme A2 was prepared by alcohol precipitation of a similar autolysate. 0.4 ml samples were used in the inhibition experiments. Activity 4.00/ml; dry weight 29 mg/ml; *I*f₃₀ = 1.6.

Enzyme A3. 1 kg of baker's yeast was mixed with 100 g solid sodium acetate and 100 ml toluene (5) and stored at 37°. After 5 days the mixture was brought to pH 4.5 with 5 *N* acetic acid and

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centrifuged; a portion was dialyzed thoroughly. 1 ml samples were used; activity 1.26/ml; dry weight 9.5 mg/ml; $I_{f_{30}} = 1.8$.

Enzyme A4 was prepared by ethanol precipitation of Enzyme A3 and dialysis. 1 ml samples in the inhibition experiments; activity 2.50/ml; dry weight 16.2 mg/ml; $I_{f_{30}} = 1.9$.

Enzyme A5. Invertase was liberated from suspension 1 with papain (6). The filtrate was precipitated with an equal volume of ethanol; the precipitate was dissolved in water and dialyzed. 1 ml samples; activity 5.60/ml; dry weight 35.2 mg/ml; $I_{f_{30}} = 1.9$.

Enzyme A6 = Enzyme A5, diluted 1:2.

Enzyme A7. 1 kg baker's yeast was mixed with 200 g solid sodium acetate and 150 ml toluene. After 3 days at 37° the mixture was filtered and the filtrate dialyzed. 1 ml samples; activity 2.12/ml; dry weight 9.5 mg/ml; $I_{f_{30}} = 2.6$.

Enzyme A8 was prepared from Enzyme A1 by absorption on aluminium hydroxide, elution with sodium phosphate and dialysis. 1 ml samples; activity 0.50/ml; dry weight 1.61 mg/ml; $I_{f_{30}} = 3.7$.

Enzyme A9. This solution was prepared from Enzyme A1 by fractional absorption on aluminium hydroxide, elution with phosphate and dialysis. 1 ml samples; activity 0.84/ml; dry weight 2.32 mg/ml; $I_{f_{30}} = 4.3$.

Enzyme A10 = Enzyme A1, treated with pancreatic ribonuclease and dialyzed.

B. Preparations from brewer's yeast

Enzyme B3. Washed brewer's yeast (dry weight ca. 10 %) was mixed with 1/10 volume of toluene and left at 30°. After days the mixture was centrifuged and a portion of the extract was dialyzed. 1 ml samples for the inhibition experiments; activity 1.55/ml; dry weight 2.6 mg/ml; $I_{f_{30}} = 7.2$.

Enzyme B4 was prepared from Enzyme B3 by fractional ethanol precipitation and dialysis. 0.5 ml samples were used; activity 1.70/0.5 ml; dry weight 1.4 mg/0.5 ml; $I_{f_{30}} = 14.6$.

Enzyme B15 was prepared (7) from an autolysate of brewer's yeast by alcohol precipitation, absorption on aluminium hydroxide, elution with phosphate and dialysis. $I_{f_{18}} = 14.5$.

Enzyme B200 was prepared (1) from an autolysate of brewer's yeast by fractional ethanol precipitation, absorption on aluminium hydroxide, elution with phosphate, dialysis, absorption on activated kaolin, elution with ammonia and dialysis. $I_{f_{18}} = 200$.

C. Preparations of I-invertase

Some of the invertase solutions were converted to the corresponding I-invertases, i.e. were oxidized by iodine (8) to yield the derivatives with constant relative activity, $RA = 0.53$. As has been reported earlier, the I-invertases are much less sensitive to Ag^+ (and certain other metal ions) than the native invertase preparations. For a comparison some values for the silver inhibition of I-invertases have been plotted in Fig. 2.

I-Invertase A51 was prepared from Enzyme A5.

I-Invertase A71 was prepared from Enzyme A7.

The I-invertase solutions were dialyzed thoroughly and were free from iodide ions.

Inhibition of invertase preparations by Ag^+

The inhibition of the invertase preparations from baker's yeast by increasing concentrations of Ag^+ is shown in Table 1 and in Fig. 2 where RA is plotted versus $\log [Ag_{tot}]$. ($[Ag_{tot}]$ is the silver ion concentration calculated from the amount of $AgNO_3$

Table 1. Silver inactivation at pH 5.3 of various invertase preparations from baker's yeast.

Invertase No. ... If ₃₀ ...	Suspension 1		A1	A2	A3	A4	A5	A6	A7	A8	A9
	1 ml	0.6 ml									
		0.32	1.3	1.6	1.8	1.9	1.9	1.9	2.6	3.7	4.3
Ag _{tot}	Relative activity (RA)										
5 × 10 ⁻⁷							0.90				
10 ⁻⁶							0.75	0.82	0.96	1.00	0.93
2 × 10 ⁻⁶							0.42	0.36	0.89	0.65	0.58
3 × 10 ⁻⁶							0.21	0.23	0.59	0.38	0.25
4 × 10 ⁻⁶									0.32		
5 × 10 ⁻⁶		1.00	1.00	0.98		1.00	0.07	0.10	0.19	0.11	0.07
7 × 10 ⁻⁶									0.10		
10 ⁻⁵		0.88		0.82	0.87			0.03	0.04		
1.2 × 10 ⁻⁵						0.88					
1.5 × 10 ⁻⁵		0.20	1.00	0.31	0.33	0.69					
1.7 × 10 ⁻⁵						0.36					
1.9 × 10 ⁻⁵						0.21					
2 × 10 ⁻⁵	1.00		0.86	0.06	0.08	0.16		0.02			
2.5 × 10 ⁻⁵	0.75		0.58								
3 × 10 ⁻⁵	0.59		0.29								
4 × 10 ⁻⁵	0.27										
5 × 10 ⁻⁵			< 0.02								

added.) For a comparison the inhibition curve of the highly purified enzyme B200 is also plotted in Fig. 2. It appears from the table and figure that the invertase preparations from baker's yeast, which all have a low specific activity, separate into two groups: one contains enzyme preparations (A5–A9) which are inhibited in the range $[Ag_{tot}] = 10^{-7}$ – 10^{-5} , and the other contains enzymes (Suspension 1, Enzymes A1–A4) which are practically not inhibited at $[Ag_{tot}] = 10^{-5}$ but completely inhibited at

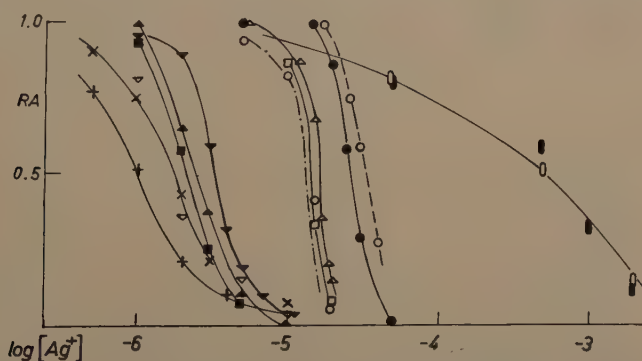


Fig. 2. Inhibition curves at pH = 5.3; relative activity (RA) plotted versus $\log [Ag_{tot}]$. ○ ---- Suspension 1, 1 ml; ○ - - - - Suspension 1, 0.6 ml.

- Enzyme A1, ○ Enzyme A2, □ Enzyme A3,
- △ Enzyme A4, × Enzyme A5, ▽ Enzyme A6,
- ▽ Enzyme A7, ▲ Enzyme A8, ■ Enzyme A9,
- + Enzyme B200, 0 Enzyme A51, ● Enzyme A71.

Table 2. Silver inactivation at pH 5.3 of various invertase preparations from brewer's yeast.

Invertase No. ... If ...	B3 If ₃₀ = 7.2	B4 If ₃₀ = 14.6	B15 If ₁₈ = 14.5	B200 If ₁₈ = 200
Ag _{tot}	Relative activity (RA)			
5 × 10 ⁻⁷	0.91	0.86	0.90	0.77
10 ⁻⁶	0.81	0.62	0.70	0.51
2 × 10 ⁻⁶	0.31	0.26	0.54	0.21
3 × 10 ⁻⁶	0.14		0.37	
4 × 10 ⁻⁶				0.10
5 × 10 ⁻⁶	0.04		0.05	
10 ⁻⁵				0.05

[Ag_{tot}] = 10⁻⁴. A third group contains the I-invertases which are incompletely inhibited even at [Ag_{tot}] = 10⁻³; the I-invertases have no free HS-groups and, consequently, are inhibited only very weakly by Ag⁺ (8).

The enzyme preparations of the second group obviously contain fairly large amounts of impurities which have a very high affinity for the silver ion. This also explains the fact that with enzyme preparations of this kind the inhibition curve will vary strongly with the amount of enzyme used in the experiments (cf. the experiments with Suspension 1) whereas in experiments with highly purified invertase the inhibition curve is found to be nearly independent of the enzyme concentration. This shows that the amounts of Ag⁺ bound by the enzyme itself are small compared to [Ag_{tot}].

Generally most of the silver-binding impurities can be removed by means of conventional purification procedures. Sometimes, however, crude autolysates, e.g. Enzyme A7, of baker's yeast contain only very small amounts of silver-binding substances, and preparations of the same degree of purity as calculated from activity and dry weight, e.g. solutions A4 and A5, may behave very differently in the presence of silver ion. Since all preparations A originated from the same baker's yeast it appears that the conditions of enzymatic liberation of the enzyme during autolysis greatly influence the sensitivity of the autolysates toward Ag⁺.

Table 2 and Fig. 3 show that all preparations from brewer's yeast, including the

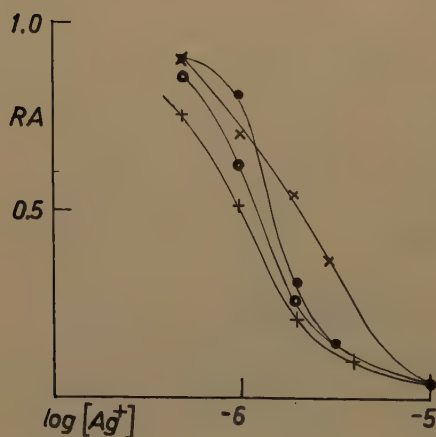


Fig. 3. Inhibition curves at pH = 5.3; relative activity (RA) plotted versus log [Ag_{tot}]. •, Enzyme B3; ×, Enzyme B15; o, Enzyme B4; +, Enzyme B200.

crude autolysate B3, are inhibited practically completely at Ag^+ concentrations $<10^{-5}$. It appears that the silver-binding impurities common in the preparations from baker's yeast are mainly absent in autolysates and purified preparations from brewer's yeast. It may be that they occur only in small quantities in the brewer's yeast used in the experiments or that they are converted during autolysis into products of low affinity for silver ions.

It was found that treatment of the preparations from baker's yeast with papain considerably increased the sensitivity of the invertase toward Ag^+ . The experiments appeared to support the assumption that the silver-binding substances may be converted enzymatically, possibly by proteolysis, to substances having only a weak affinity for Ag^+ . Since it is by no means clear how a strongly silver-binding substance could, by the action of a proteolytic enzyme, be transformed into substances with a very low silver-binding capacity, we tried to determine the nature of the silver-binding impurities in the crude invertase solutions and the nature of their enzymatic conversion.

Preliminary characterization of the silver-binding substances in invertase solutions

The Ag inhibition curve of Enzyme A2 (curve *a* in Fig. 4) shows that the amount of enzyme used (11.6 mg in 100 ml assay mixture) contains some contaminating substance(s) capable of binding with a very high affinity about 8×10^{-7} equiv. Ag at pH 5.3.

A portion of Enzyme A2 was heated at pH = 5 and 70°C for 30 min; the enzymatic activity of the solution was completely destroyed. Inversion experiments were now performed with Enzyme A6 and Ag^+ at pH 5.3 (cf. Table 1 and curve *b* in Fig. 4) with the addition of the inactivated enzyme A2 (Table 3). From these experiments one may calculate the amount of Ag bound at pH 5.3. For instance, the addition of 2.9 mg inactivated A2 causes the RA of enzyme A6 at $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6} N$ to rise from 0.10

Table 3. Relative activity (RA) of enzyme A6 at $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6}$ and different pH-values in the presence of inactivated enzyme A2.

pH	mg inactivated A2 added	RA
4.5	—	0.60
	2.9	0.85
4.8	—	0.33
	2.9	0.23
5.3	—	0.10
	2.9	0.23
5.65	5.8	0.74
	—	0.03
5.95	2.9	0.11
	5.8	0.53
6.58	—	~ 0.01
	2.9	0.04
	5.8	0.30
	—	< 0.01
	5.8	0.08

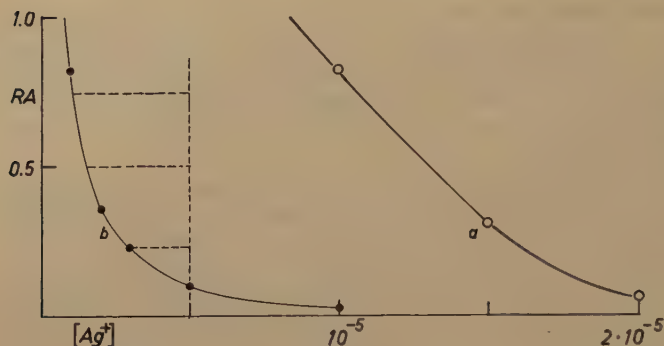


Fig. 4. *a*, inhibition curve of Enzyme A2; *b*, inhibition curve of Enzyme A6. The dashed lines demonstrate calculation of amount of Ag bound (see text).

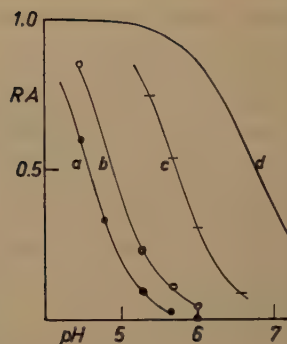


Fig. 5. Inhibition-pH-curves of Enzyme A6: *a*, $[Ag_{tot}] = 5 \times 10^{-6}$; *b*, the same in the presence of 2.9 mg inactivated Enzyme A2; *c*, the same in the presence of 5.8 mg inactivated Enzyme A2; *d*, activity-pH-curve of Enzyme A6 (no inhibitor).

to 0.23. From the left part of Fig. 4 it is seen that this corresponds to the binding of about 2×10^{-7} equiv. Ag. Similarly we find that the addition of 5.8 mg inactive A2 rises RA from 0.10 to 0.74, corresponding to the binding of about 4×10^{-7} equiv. Ag. Thus we may conclude that heating of Enzyme A2 at 70° for 30 min does not measurably decrease its Ag-binding capacity.

The protecting action of inactivated Enzyme A2 on the Ag inhibition of enzyme A6 at various pH values is seen in Fig. 5. It appears that the curves (*b*, *c*) showing the inhibition in the presence of, respectively, 2.9 and 5.8 mg of inactivated Enzyme A2, are approximately parallel to the inhibition curve *a* without protecting substance, and approximately parallel to the alkaline branch, *d* of the activity-pH-curve. In other words, the two curves *b* and *c* coincide approximately with curves representing the inhibition of Enzyme A6 by concentrations, $[Ag_{tot}]$, lower than 5×10^{-6} N, viz. 3×10^{-6} and 10^{-6} N, respectively. This means that the silver-binding capacity of the substance(s) in the inactivated Enzyme A2 is only weakly dependent on pH. One explanation would be that silver is bound by sulphhydryl groups.

The solution of the inactivated Enzyme A2 was dialyzed for three days against distilled water that was changed frequently. The dialyzed solution was tested for protecting action on the Ag inhibition of Enzyme A6 (Table 4). The experiment shows that Ag^+ is bound by non-dialyzable substances.

Oxidation of the inactivated Enzyme A2 by iodine was carried out as described earlier (8): 5 ml solution + 1 ml 0.0039 N iodine solution. The product was dialyzed to remove iodide ions and other low molecular substances that might have been formed. Table 4 shows that the protecting action has decreased somewhat; however, from Fig. 4 it can be seen that 5.8 mg of the oxidized and dialyzed preparation bind about 3.5×10^{-7} equiv. Ag, compared to 4×10^{-7} equiv. for the non-oxidized solution. This appears to rule out the possibility that the binding of Ag^+ by substances in the solution A2 is mediated chiefly by HS groups.

Table 4. Protecting effect of inactivated enzyme A2 after various treatments.
 $[Ag_{tot}] = 5 \times 10^{-6} \text{ N}$, pH = 5.3.

In all experiments the addition corresponds to 5.8 mg of the inactive preparation A2.

Addition	RA
No addition	0.10
Prep. A2, no treatment	0.70
dialyzed	0.70
oxidized with iodine, dialyzed	0.50
after hydrolysis in 3 N H_2SO_4 , 100°, 8 h	0.08
after hydrolysis in 0.25 N H_2SO_4 , 30°, 20 h	0.17
after hydrolysis in N NaOH, 30°, 20 h	0.09
treated with papain: 0 hours	0.74
17 hours	0.24
3 days	0.15
26 days	0.07

Table 4 shows further that acid as well as alkaline hydrolysis, even under rather mild conditions, completely abolishes the silver-binding power of the inactivated solution A2. This does not support an assumption that HS-groups are responsible for the binding of silver; hydrolysis of, e.g., egg albumin does not decrease the silver-binding capacity.

The action of papain (Merck, 1:350) on the substances in the inactivated solution A2 was investigated. The solution was mixed with an equal volume of 1 % papain solution and some toluene and stored at 30°, pH = 5. The amount of papain present in the inhibition experiments has no measurable protecting action. It is seen from Table 4 that treatment with papain abolishes the protecting action of the preparation A2 completely. At first sight this would seem to intimate that the protecting substances in A2 are proteins. It is striking, however, that the action of the very large amounts of papain used is very slow.

Since invertase itself is not damaged by papain, the action of this enzyme on an active but rather crude invertase solution was tested. Enzyme A3 was used for these experiments; it is seen from Table 1 and from Fig. 6 that this solution contains rather large amounts of silver-binding impurities.

(a) To 1 l enzyme A3 was added 1 g papain and some toluene. The mixture was kept at 30°C for 20 days. A portion was dialyzed = Enzyme A32. 1 ml samples were used for the inhibition experiments; activity 1.22/ml; dry weight 7.78 mg/ml; $If_{30} = 1.9$, curve *b* in Fig. 6.

(b) Another portion of the enzyme-papain mixture was kept at 30° for 110 days and then dialyzed = Enzyme A33. 1 ml samples for the inhibition experiments; activity 0.92/ml; dry weight 6.67 mg/ml; $If_{30} = 1.7$; curve *c* in Fig. 6.

In Fig. 6 the inhibition curve (dashed curve *d*) of the highly purified Enzyme B200 has been plotted for a comparison. The four inhibition curves show that nearly all the silver-binding impurities of Enzyme A3 have been destroyed by the papain. However, the activity of the dialyzed, papain-treated solutions per g dry weight, has not changed appreciably. This must mean either that the products of the papain action are not dialyzable or that the silver-binding impurities in Enzyme A3 are only a very small fraction of the total dry weight of the solution.

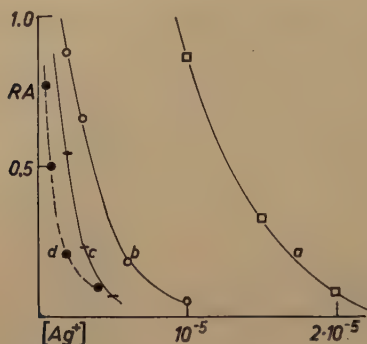


Fig. 6. Action of papain on Enzyme A3: *a*, inhibition curve of Enzyme A3 (not treated); *b*, Enzyme A3 treated with papain for 20 days; *c*, Enzyme A3 treated with papain for 110 days; *d*, inhibition curve of Enzyme B200.

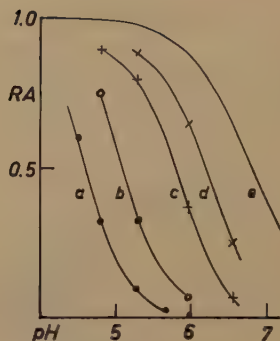


Fig. 7. Inhibition-pH-curves of Enzyme A6: *a*, $[Ag_{tot}] = 5 \times 10^{-6}$; *b*, the same in the presence of 0.36 mg A34; *c*, the same in the presence of 0.54 mg A34; *d*, the same in the presence of 0.72 mg A34; *e*, activity-pH-curve of Enzyme A6 (no inhibitor).

With the idea of characterizing the silver-binding substance(s) in Enzyme A3, a 200 ml portion of this solution was precipitated with 30 g trichloroacetic acid. The washed precipitate was suspended in water and brought into solution by careful addition of sodium hydroxide. After dialysis the total dry weight of the solution (preparation A34) was 0.20 g or about 10 % of the dry weight of the precipitated volume of Enzyme A3. The preparation A34 had no invertase activity. The protecting action of the solution against the Ag^+ inhibition of Enzyme A6 was determined as described above. It appears from Fig. 7 that the action of the substances precipitated by trichloroacetic acid is qualitatively the same as that of, e.g., the thermoinactivated solution A2 (Fig. 5); the binding of Ag^+ does not vary appreciably with pH. A comparison of Figs. 5 and 7 shows that, quantitatively, the action of solution A34 is much (about 10 times) stronger than that of the inactivated solution A2. At pH 5.3 and $[Ag_{tot}] = 5 \times 10^{-6}$ the RA is raised by 0.54 mg A34 from 0.10 to 0.80. This means that about 4×10^{-7} equiv. Ag have been bound, i.e. about 7.4×10^{-7} equiv. Ag/mg.

Electrometrical silver determinations

The Ag-binding capacity of some of the preparations mentioned above has also been determined electrometrically; the method has been described in earlier papers (9, 10). The volume of the solutions in the electrode vessels was 20 ml, $[Ag_{tot}] = 5 \times 10^{-4}$ N.

Preparation A 34, i.e. the trichloroacetic acid precipitate from Enzyme A3, was tested; Fig. 8 shows that this preparation binds Ag^+ strongly at all pH values investigated. The binding capacity increases somewhat with increasing pH. Since the curves of Fig. 8 are not straight lines it can be concluded that at least part of the bound Ag is relatively loosely bound. At pH = 5.3 1.8 mg substance binds about 4×10^{-6} equiv. of Ag^+ or about 2.2×10^{-6} equiv./mg substance. This value is considerably higher than that calculated from the inhibition experiments, but it should be remembered that the total Ag concentration is 100 times higher in the electrometric experiments.

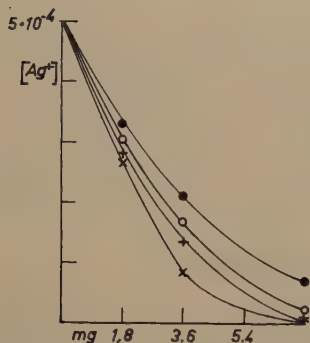


Fig. 8. Electrometric determination of $[Ag^+]$ in the presence of preparation A34 at different pH values: ●, pH = 4.5; ○, pH = 5.3; +, pH = 5.95; ×, pH = 6.7. $[Ag_{tot}] = 5 \times 10^{-4} N$, volume 20 ml.

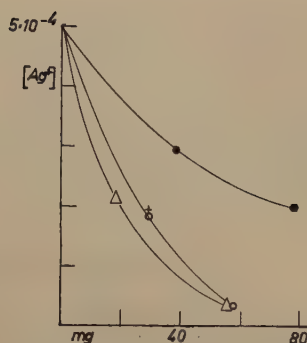


Fig. 9. Electrometric determination of $[Ag^+]$ in the presence of various invertase preparations: ○, Enzyme A2; +, heat-inactivated Enzyme A2; Δ, Enzyme A3; ●, papain-treated Enzyme A32. $[Ag_{tot}] = 5 \times 10^{-4} N$, volume 20 ml, pH = 5.3.

Table 5. Decrease of Ag^+ in the presence of various preparations, measured electrometrically.

Preparation	mg added	pH	$[Ag_{free}] \times 10^4$
Enzyme A2	29.0	—	5.00
		4.50	2.46
		5.35	1.85
		5.95	1.52
		6.70	1.02
	58.0	4.50	0.81
		5.35	0.33
		5.95	0.17
		6.70	0.14
Enzyme A2, heated at 70°, 30 min.	29.0	4.50	2.69
		5.35	1.94
		5.95	1.55
		6.70	1.10
Enzyme A3	19.0	5.30	2.02
		5.95	1.40
		6.70	1.29
	47.5	5.30	0.34
Enzyme A32 (treated with papain)	38.9	4.50	3.96
		5.30	2.94
	77.8	4.50	2.29
		5.30	1.99
		5.95	1.38
		6.70	1.09

Electrometric determination of the silver binding by some other preparations are compiled in Table 5, and the measurements at pH = 5.3 are shown in Fig. 9. In agreement with the results of the inhibition experiments it is found that the heat-inactivated preparation A2 binds Ag^+ to about the same extent as the enzymatically active Enzyme A2. It is also found that Enzymes A2 and A3 have about the same Ag-binding capacity whereas the papain-treated Enzyme A32 binds Ag^+ much more weakly; this is also in good agreement with the results of the inhibition experiments.

Nature of the silver-binding impurities

In spite of the experiments on the action of papain it seemed improbable that the binding of Ag^+ by impurities in the invertase solutions could be due mainly to the presence of silver-binding proteins. Since the preparation A34, precipitated with trichloroacetic acid, was especially rich in silver-binding substances, a plausible explanation would be that nucleic acids are mainly responsible for the protecting action on invertase. It was found that preparation A34 and the crude invertase solutions from baker's yeast were comparatively rich in combined phosphoric acid.

The protecting action of yeast nucleic acid (commercial preparation) on the inhibition of yeast invertase by Ag^+ was investigated: Enzyme A6, pH = 5.3, $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6} N$. Substrate solution, buffer and silver solution were mixed and brought to 30°. The nucleic acid solution was added; if not otherwise stated the enzyme A6 was added 1 min after the addition of the nucleic acid. Table 6 shows that yeast nucleic acid binds considerable amount of silver strongly; the complexing of Ag^+ by yeast nucleic acid is instantaneous and independent of preincubation. (The action of the dialyzed nucleic acid solution is somewhat stronger than that of the solution before dialysis, possibly because the non-dialyzed solution contains degradation products of lower affinity for Ag^+ .)

Experiments summarized in Table 7 show that acid or alkaline hydrolysis of yeast nucleic acid abolishes its protecting action. The silver-binding capacity decreases

Table 6. Protecting action of yeast nucleic acid on the silver inactivation of enzyme A6, pH = 5.3, $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6}$.

Addition	RA
None	0.07
<i>Non-dialyzed solution</i>	
Nucl. acid, 0.61 mg	0.16
0.61 mg; Ag^+ , nucl. acid and enzyme, incub. 70 min	0.13
0.61 mg; Ag^+ and nucl. acid, incub. 60 min	0.16
1.22 mg	0.49
6.12 mg	1.00
<i>Dialyzed solution</i>	
0.41 mg	0.15
0.82 mg	0.48
1.03 mg	0.70
2.06 mg	0.97

Table 7. Protecting action of yeast nucleic acid solution (dialyzed) after hydrolysis:
Enzyme A6, pH = 5.3, $[Ag_{tot}] = 5 \times 10^{-6}$.

Nucleic acid, mg	Hydrolysis			RA
—	—	—	—	0.07
1.03	No treatment	—	—	0.70
	<i>N</i> H ₂ SO ₄	100°,	3 h	0.07
5.15	<i>N</i> H ₂ SO ₄	100°,	3 h	0.27
1.03	<i>N</i> H ₂ SO ₄	20°	24 h	0.15
	<i>N</i> NaOH	100°	3 h	0.07
	<i>N</i> NaOH	20°	5 min	0.40
	<i>N</i> NaOH	20°	30 min	0.14
	<i>N</i> NaOH	20°	60 min	0.09
	<i>N</i> NaOH	20°	24 h	0.06
	0.5 <i>N</i> H ₂ SO ₄	20°	5 min	0.57
	0.5 <i>N</i> H ₂ SO ₄	20°	10 min	0.53
	0.5 <i>N</i> H ₂ SO ₄	20°	30 min	0.53
	0.5 <i>N</i> H ₂ SO ₄	20°	1 h	0.42
	0.5 <i>N</i> H ₂ SO ₄	20°	2 h	0.22
	0.5 <i>N</i> H ₂ SO ₄	20°	4 h	0.21
	0.5 <i>N</i> H ₂ SO ₄	20°	7 h	0.13
	Water, pH ~ 4	100°	1 h	0.29
	Water, pH ~ 4	100°	3 h	0.16

Table 8. Protecting action of yeast nucleic acid treated with papain. Enzyme A6,
0.70 mg nucleic acid, pH = 5.3, $[Ag_{tot}] = 5 \times 10^{-6}$.

Reaction time, h	RA
0	0.38
5	0.34
23	0.21
71	0.11
122	0.07

fairly rapidly in 0.5 *N* H₂SO₄ or *N* NaOH at 20°C, and even boiling of the nucleic acid solution (pH about 4) for a few hours is enough. These results obviously strongly support the conclusion that the protecting action on the silver inhibition of impurities in yeast invertase solutions can be ascribed mainly to the presence of yeast nucleic acid in the enzyme preparations. It has to be shown, however, that papain has an action on the nucleic acid preparation comparable to that on the protecting substances in the invertase solutions (cf. Table 4).

10 ml nucleic acid solution was mixed with 5 ml papain solution (pH = 5) and stored at 30°. Table 8 shows that papain destroys the protecting action of the nucleic acid solution. The action of papain is slow, just as its action on the protecting substances in the invertase solutions. Obviously the papain preparation contains traces of nuclease.

The complexing of Ag⁺ by nucleic acids will be the subject of a forthcoming publication.

SUMMARY

Crude or partly purified yeast invertase solutions may contain impurities which bind silver ions strongly and thus more or less protect the enzyme against inhibition by these ions. The amount of protecting substances in an invertase solution depends upon the type of yeast used, the method for liberating invertase from the insoluble cell structures and, of course, on the purification procedures applied. Autolysates of baker's yeast generally contain large amounts of protecting substances whereas autolysates of brewer's bottom yeast usually contain only small amounts.

The protecting substances are high molecular and tolerably thermostable. However, on boiling the solutions for hours the protecting activity decreases. Acid or alkaline hydrolysis abolishes the protecting activity rapidly and almost completely. Comparative experiments show that the natural protecting substances in invertase solutions are mainly to be identified with yeast nucleic acid. The polynucleotide obviously has a much higher affinity for silver ions than have its degradation products.

The experimental part of this investigation has been carried out by Mrs. Ebba Willstaedt whose assistance is gratefully acknowledged.

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Starch degradation by the α -amylases

V. Limit dextrins of salivary amylase

By GUNNAR AHNSTRÖM

With 7 figures in the text

The glucose residues of starch are united by α -glucosidic linkages. The amylose component is considered to have unbranched molecules with 1,4-linkages exclusively; however, the existence in amylose of a small number of other linkages does not seem to be excluded (1). In the amylopectin there are about 95 % α -1,4-glucosidic linkages and about 5 % α -1,6-linkages, the latter corresponding to branching points of the molecules.

The ordinary amylases hydrolyze, as far as is known at present, only α -1,4-linkages; this is true for β -amylase and for the various α -amylases (salivary, pancreatic, malt, mold and bacterial α -amylases). Polysaccharides with only α -1,4-linkages (amylose and its degradation products) are completely transformed to fermentable sugars by all amylases: β -amylase yields maltose and small amounts of maltotriose, the latter from chains with an uneven number of glucose units; the α -amylases give maltose as the chief degradation product but also small amounts of glucose and possibly maltotriose.

The action of the amylases on branched molecules (amylopectin) is strongly influenced by the 1,6-linkages of the substrates. The action of β -amylase which attacks the substrate molecules from the free non-reducing endgroups is topped at a distance of 1–2 glucose units from the branching points. This enzyme, therefore, will form from amylopectin about 50 % maltose and a high-molecular, amylopectin-like limit dextrin (" β -dextrin"). The α -amylases, which attack 1,4-linkages independent of endgroups, i.e. also *between* branching points of the amylopectin molecules, will "dextrinize" this substrate completely, i.e. convert it into a mixture of maltose, glucose and limit dextrins (generally about 15 %) with 3 or more glucose units. Obviously, the 1,6-linkages of the substrate not only are not hydrolyzed by the enzymes, but they also prevent or at least delay the hydrolysis of a certain number of 1,4-linkages in close vicinity of the branching points.

A survey of the limit dextrins that may be formed from amylopectin by the α -amylases has been given by Myrbäck (2). Limit dextrins with more than one 1,6-linkage per molecule should be exceptions; this is clear from a comparison of the number of 1,6-linkages in amylopectin and the normal yield of limit dextrins.

Among theoretically possible limit dextrins there are 15 hexasaccharides; however, 11 of these probably can be attacked already by β -amylase with the liberation of maltose units, and it must be concluded that these dextrins are hydrolyzed also

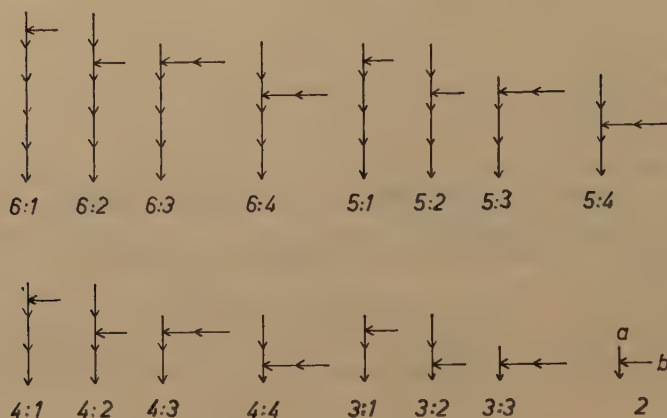


Fig. 1. Limit dextrins with 2-6 glucose units.

by the α -amylases. The four remaining hexasaccharides are schematically shown in Fig. 1 (6:1-6:4). It seems probable that they, or at least the three first (6:1-6:3) can be attacked by α -amylases, since they contain rather long chains with 1,4-linkages. (In Fig. 1 the glucose unit is represented by an arrow, the point being the (potentially) reducing group, i.e. carbon atom 1. The α -1,4-linkage is indicated by a point-tail attachment of the arrows, the α -1,6-linkages by a point-shaft attachment.)

Six of the ten possible pentasaccharides with one 1,6-linkage will probably yield maltose with β -amylase and will certainly be attacked by the α -amylases. The remaining four (5:1-5:4 in Fig. 1) may be thought to appear among the limit dextrins of the α -amylases. The same should be true of the four tetrasaccharides 4:1-4:4 (out of six possible tetrasaccharides with one 1,6-linkage), the three possible trisaccharides 3:1-3:3, and the disaccharide, isomaltose (Figs. 1, 2). Thus it seems a reasonable assumption, that the limit dextrins of the α -amylases must be sought among the di-, tri-, tetra- and pentasaccharides, depicted in Fig. 1.

Concerning the structure of the α -amylase limit dextrins, supposed to be stable to further action of the amylase in question, different opinions have been expressed by various authors. Meyer *et al.* (3) assumed that the only true limit dextrin of the α -amylases is isomaltose, i.e. they assume that all 1,4-linkages of starch dextrins can be hydrolyzed by the α -amylases, irrespective of their position in relation to the 1,6-linkages. However, several facts are inconsistent with this assumption. For instance the total yield of limit dextrin from starch would be only about 8%, whereas at least 15% is found (provided that the enzyme is not contaminated by "limit dextrinases"). Furthermore, analyses of various limit dextrin fractions, by paper chromatography and other methods, have shown the presence of large amounts of tetra- and pentasaccharides, sometimes also trisaccharides, whereas isomaltose was absent or present only in trace amounts.

In the case of salivary amylase Myrbäck and Willstaedt (4) have found that a tetrasaccharide should be regarded as the lowest stable dextrin, whereas Whelan and Roberts (5) assume that a pentasaccharide, viz. the one designated 5:4 in Fig. 1 is the true stable dextrin.

It seems that in the discussion of the stable dextrins, there are a few points which

are sometimes more or less overlooked. For instance, it does not appear necessary to assume that all α -amylases should produce the same stable dextrin(s), nor that a certain α -amylase should yield only one truly stable dextrin. Since it is generally assumed that in amylopectin the glucose units are practically randomly distributed on chains of different lengths (6), the products of the primary rapid action of α -amylases on amylopectin (starch) ("liquefaction, dextrinization") will be a mixture of dextrins containing, on the average, 9 glucose units per molecule. If we disregard the few molecules containing more than one 1,6-linkage, the "primary α -dextrins" will contain *one* chain with a reducing endgroup, and *two* chains with non-reducing endgroups. The number of glucose units in these chains will vary around certain mean values. It appears possible that, when the enzyme splits off maltose or glucose units from the primary dextrins (the rate of hydrolysis decreasing with the number of glucose units of the substrate) the structure of the end-product (stable dextrin) may be different depending on the rather random distribution of the glucose units on the different chains of the primary dextrin. For instance, a primary dextrin with an even number of glucose units in the reducing end-chain may yield another stable dextrin than a similar primary dextrin with an uneven number of units in the reducing end-chain.

That all α -amylases have not the same action on starch, is evident already from the shape of the curves showing the degree of hydrolysis as a function of reaction time. In the case of malt α -amylase this curve shows two clearly separated reactions: One rapid phase ("dextrinization") comprising the hydrolysis of about 17 % of the glucosidic linkages of the starch, and a slow "saccharification", leading finally to the hydrolysis of 40–45 % of the linkages (7). In the case of salivary amylase on the other hand, the curve is continuously bent, up to a degree of hydrolysis of approximately 40 % (i.e. a yield of maltose around 80 %). After that follows (cf. Table 1) a slow increase of the degree of hydrolysis whereby glucose possibly also maltose is split off (8).

An important question in the discussion of the stable dextrins is, of course, the homogeneity of the enzyme preparations used in the hydrolysis experiments. Meyer, Duckert and Fischer (9) have found that raw saliva has the same action on starch as has crystalline salivary amylase; this would mean that no other carbohydrases of importance in connection with starch hydrolysis are present in saliva. It seems, however, that the reaction times used by the authors were too short to allow the exclusion of differences in the later slow phase of the hydrolysis. Maltase is present at least in certain samples of saliva (10). However, heated samples of saliva (10) or highly purified preparations of salivary amylase which have no action on maltose, nevertheless form glucose from starch, intimating that salivary amylase is able to split off glucose from certain higher oligosaccharides (limit dextrins). This secondary saccharification proceeds with continuously decreasing velocity for a very long time (cf. Table 1) and a definite "limit of saccharification" cannot be stated with certainty. Obviously, if the degree of hydrolysis is determined exclusively by the increase of the reducing power, erroneous values of the saccharification limit may be obtained, especially if the amylase preparation used contains maltase. Therefore, in this investigation the hydrolysis has been followed not only by determination of reducing sugar, but also by determinations of fermentable sugar, glucose and maltose being determined separately (11) and by paper chromatography.

As mentioned above, in earlier investigations in this laboratory a tetrasaccharide was found to be the chief component of the stable (or practically stable) limit dextrins

Table 1. Course of starch degradation.

Reaction time days	% linkages ruptured
0.05	32.3
1	38.0
4	42.9
14	51.8
26	56.6
52	64.3
200	73.8

of salivary amylase, whereas Whelan and Roberts have found that a pentasaccharide (5:4) should be regarded as the true limit dextrin. This question has now been reinvestigated, the work being concentrated on oligosaccharides with not more than 5 glucose units, arising from the action of salivary amylase on starch. For separation of the products the method of Whistler and Durso (12) was applied, i.e. absorption on a carbon-celite column and graded elution with suitable solvent mixtures. This method in more or less modified form has been extensively used for separations of substances of similar nature, e.g. by Lindberg (13).

For determination of the structure of the various oligosaccharides, two methods were used:

(a) *Oxidation by periodate*. Ahlberg (14), Neumüller and Wasseur (15) and Hultin (16) have studied this reaction, using oligosaccharides with different types of glucosidic linkages, and have found, *inter alia*, that by oxidation at pH values below 1 the position of the 1,6-linkages in low molecular starch limit dextrins can be determined.

(b) *Action of amylase*. The various limit dextrin fractions were treated for long time with large amounts of salivary amylase. The course of hydrolysis (if any) was followed by paper chromatography. Parallel experiments were conducted with the same fractions after oxidation of the reducing end-group to a carboxyl. The oxidation was carried out as described by Myrbäck and Nycander (17).

Preparation of limit dextrins

170 g (dry weight) Lintner starch (prepared in the usual way from potato starch with hydrochloric acid) was dissolved in 3.8 l boiling water. After cooling to 30° pH was adjusted to 6.9 and 200 ml filtered saliva was added together with some toluene. The mixture was kept in a water thermostat at 30°. The increase of the reducing power was determined according to the Willstätter-Schudel method, and the relative number of glucosidic linkages ruptured was calculated (Table 1). The later stages of the reaction ("saccharification") was also followed by paper chromatography. Solvents were either butanol-pyridine-water (6:4:3) or ethyl acetate-acetic acid-water (60:17:17.5). Glucose, maltose and, when available, higher saccharides, were run as standard substances on each chromatogram. R_F -values (18) were determined and used for a preliminary identification of the various sugars.

The paper chromatograms showed a gradual increase of the amounts of maltose, glucose and a tetrasaccharide fraction whereas the amounts of maltotriose and dextrins with 5 or more glucose units decreased.

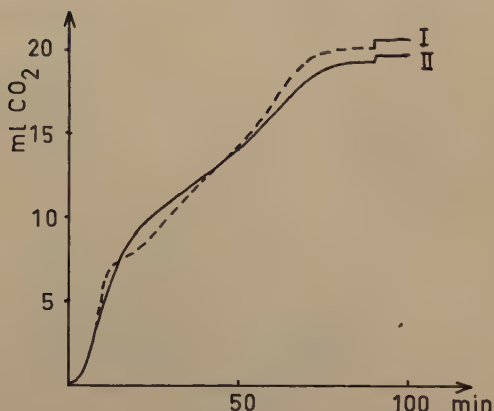


Fig. 2. Fermentation curves: I saliva-treated starch, 2 ml sample; II 40 mg glucose + 47.5 mg maltose.

After 3 months the mixture was filtered and freed from toluene. The dry weight was determined. A small sample corresponding to 103 mg dry weight was fermented with baker's yeast and the evolution of carbon dioxide was measured. Comparison with parallel experiments with known amounts of glucose + maltose shows (Fig. 2) that the sample contains about 35 mg glucose and about 57 mg maltose (anhydrous), the last figure possibly including small amounts of other fermentable oligosaccharides. The amount of nonfermentable dextrans in the reaction mixture would then be about 15 %.

Obviously a rather strong hydrolysis of maltose to glucose must have taken place in the reaction mixture, i.e. the sample of saliva used has contained maltase. This means that the increase of the reducing value during the later stages of the hydrolysis is due chiefly to hydrolysis of maltose and only to a lesser extent to liberation of glucose from limit dextrans.

3 l of the reaction mixture, corresponding to 130 g starch (dry weight) was treated with 300 g baker's yeast at 30°. After the fermentation had ceased, the mixture was centrifuged, filtered and the volume was reduced to 100 ml. This solution contained 20 g dry material. The average chain length of the dextrans was 6.8.

Separation of limit dextrans

The carbon column used had the dimensions 4 × 25 cm. Equal amounts by weight of carbon (Darco G-60) and Celite were mixed dry and then mixed with distilled water to a thick slurry. Portions of about 25 ml each were filled into the column and packed by means of a vibrator.

Ethanol-water or methylethylketone-water was used for elution. The elution rate was regulated by adjusting the height over the column of the vessel containing the eluent. The eluate was taken up by means of a fraction collector in portions of about 60 ml. The total amount of sugar in the tubes was determined by means of the anthrone method in 1 ml samples.

Experiment 1

5 g of the dextrin mixture was applied to the column and eluted with methylethylketone-water. The distribution of the material in the eluate appears from Fig. 3.

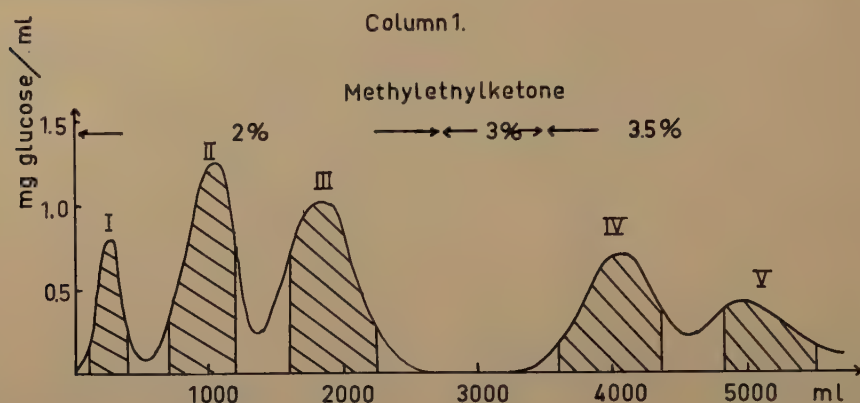


Fig. 3. Separation of limit dextrins on a carbon-celite column; elution with methylethylketone.

As indicated in this figure the contents of several tubes were mixed to yield five fractions corresponding to the peaks of the distribution curve. Samples of these fractions were concentrated in vacuo and paper chromatograms were prepared (Fig. 5).

Fraction I (0.10 g) contains only small amounts of glucose and maltose.

Fraction II (0.42 g) contains only a trisaccharide with one 1,6- and one 1,4-linkage, panose, 6- α -glycosyl-maltose, in earlier papers from this institute designated as IM-3.

Fraction III (0.60 g) contains largely IM-4, i.e. tetrasaccharides with one 1,6- and two 1,4-linkages, together with small amounts of maltotriose (M-3).

Fraction IV (0.24 g) contains chiefly limit dextrin IM-5 (pentasaccharides with one 1,6-linkages) together with maltotetraose (M-4).

Fraction V (0.27 g) was not further investigated since it presumably contains only higher limit dextrins.

If we assume that no appreciable losses of oligosaccharides with less than 5 glucose units has occurred during sorption and elution, it seems that about 50 % of the total yield of limit dextrins contains 5 or more glucose units.

Experiment 2

A limit dextrin preparation obtained by ethanol fractionation of limit dextrins produced from arrow root starch by salivary amylase was fractionated on the carbon column. The preparation has been described earlier (19) under the designation AS X. It had an average molecular weight of 1180, i.e. an average degree of polymerisation of 7. 1.5 g of the preparation was applied to the column and eluted with ethanol-water. The eluate was collected as in Experiment 1. The results can be seen in Fig. 4. The four fractions indicated in the figure were tested by paper chromatography. It appears from Fig. 6 that glucose, di- and trisaccharides were absent from all fractions, obviously because a preliminary precipitation with ethanol had been carried out.

Fraction I contains the limit dextrin tetrasaccharide IM-4 exclusively.

Fraction II: Only pentasaccharides (IM-5) are present.

Fractions III and IV contain limit dextrins with 5 and 6 glucose units.

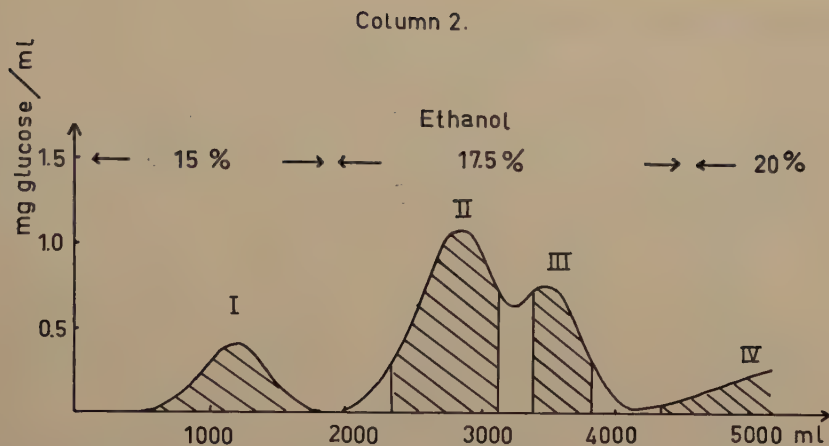


Fig. 4. Separation of limit dextrins on a carbon-celite column; elution with ethanol.

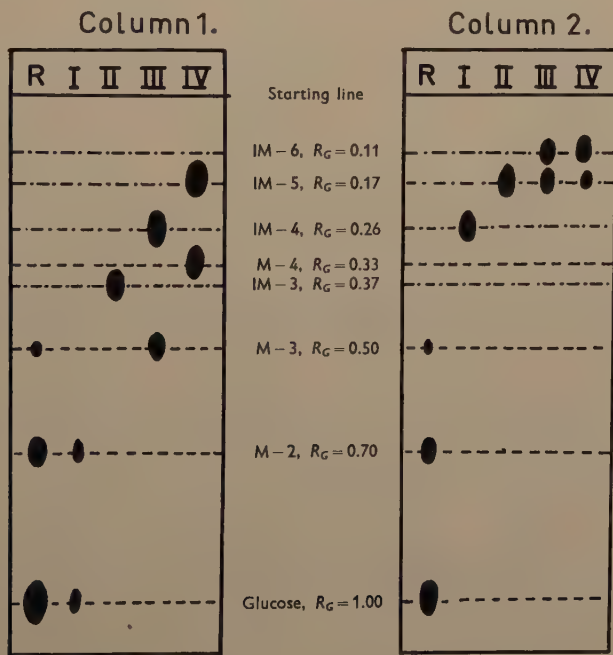


Fig. 5.

Fig. 6.

Experiment 3

The saccharides of fractions III and IV from experiment 1 could be further separated by chromatography on thick filter paper (13). About 50 mg of the substances was applied as a 10 cm long band across the strips and the chromatograms were developed with butanol-pyridine-water (6:4:3). The developed papers were dried;

Table 2. Number of glucose units in limit dextrins separated by paper chromatography (Experiment 3).

R_G of dextrin	Dextrins before hydrolysis			Dextrins after hydrolysis		Number of glucose units
	Blank, ml thiosulphate	ml sample	ml thio-sulphate	ml sample	ml thio-sulphate	
0.17	3.06	10	2.56	5	1.79	5.16
	3.06	10	2.56	5	1.75	
0.26	3.06	5	2.80	5	2.02	3.95
	3.06	5	2.79	5	2.01	
0.33	3.02	5	2.81	5	2.23	3.76
0.37	3.00	10	2.76	10	2.29	2.96
	2.99	10	2.75	10	2.28	

on each side a narrow strip was cut off and treated with silver nitrate. The various bands on the main part of the chromatogram were located in this way; they were cut out and eluted with water. The reducing power before and after hydrolysis (*N* hydrochloric acid, 100°, 3 h) was determined and the number of glucose units in the dextrins was calculated. It appears from Table 2 that the four dextrins separated contain 5, 4, 4 and 3 glucose units, respectively.

Oxidation with periodate. The tri-, tetra- and pentasaccharides from Experiment 1 were oxidized with periodate: A number of tubes were prepared containing 1 ml dextrin solution (2–4 mg sugar), 2 ml 2 *N* sulfuric acid and 1 ml 0.1 *M* potassium periodate solution. The tubes were kept in a water thermostat at 45°C. After suitable time intervals the contents of a tube was cooled and neutralized with *N* NaOH. 25 ml 0.1 *M* phosphate buffer, pH 7, was added and then 5 ml 10% solution of potassium iodide. Free iodine was titrated with 0.1 *N* thiosulfate and the number of moles

Table 3. Periodate oxidation of limit dextrins from Experiment 1.

Reaction time hours	Moles periodate consumed/mole dextrin		
	IM-3	IM-4	IM-5
0.25	6.5	—	10.0
0.50	10.7	11.0	11.2
0.75	11.5	—	—
1.00	—	12.6	14.0
1.25	11.7	—	—
1.50	—	13.3	—
2.00	12.3	—	—
2.25	—	—	15.0
2.50	—	14.2	—
2.75	12.3	—	—
3.00	—	—	15.2
3.50	—	14.2	—
4.50	—	14.2	—

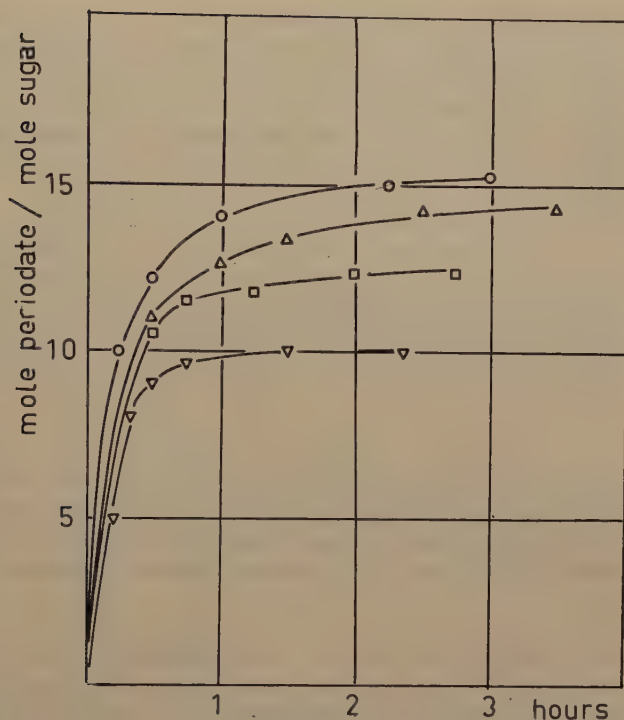


Fig. 7. Periodate oxidation:
 ▽ maltose, □ IM-3, △ IM-4,
 ○ IM-5.

periodate consumed per mole sugar was calculated (Table 3). The values and corresponding values for maltose are plotted in Fig. 7.

The periodate oxidation of sugars is a two-phase reaction: The first phase, the "normal oxidation" is rapid and corresponds to the oxidative rupturing of linkages between adjacent carbon atoms, both carrying free hydroxyl groups. This phase is followed by an "overoxidation" the velocity of which is determined by the acidity of the solution (15). In strongly acid solutions the velocity is nearly as high as that of the "normal oxidation". The extent of the overoxidation depends on the nature and position of the glycosidic linkages. At pH 0 a saccharide with only 1,4-linkages consumes 5 moles periodate per glucose unit. If a saccharide contains 1,6-linkages like the stable dextrans, the overoxidation is stopped at the 1,6-linkage. This means that one molecule of maltose consumes 10 moles of periodate whereas one mole of isomaltose consumes only 6. The corresponding values for the various limit dextrans with one 1,6-linkage can be calculated on the basis of the value for isomaltose. Addition of one glucose unit to the reducing group of isomaltose will increase the periodate consumption by 5 moles, i.e. the trisaccharide panose (3:1, Fig. 1) will consume 11 periodate molecules per mole sugar. On the other hand addition of one glucose unit to the 4-position of glucose residue (a) of isomaltose (Fig. 1), resulting in the trisaccharide 3:2, does not increase the consumption of periodate, whereas the addition of one glucose unit to the 4-position of glucose residue (b), resulting in the trisaccharide 3:3, increases the periodate consumption from 6 to 7 molecules per dextrin molecule. The experiments (Table 3, Fig. 7) clearly show that the trisaccharide IM-3

from Experiment 1 has the structure 3:1. This sugar was first isolated by Myrbäck and Ahlborg (20) from a starch dextrin fraction obtained from corn starch with malt amylase. Using the classical method of methylation and hydrolysis the presence of one 1,6- and one 1,4- α -glucosidic linkage was established. Subsequently the sugar has been obtained as a product of transglycosylation (21) and has been called panose. It has been obtained in crystalline form and its structure (4-O- α -isomaltopyranosyl-D-glucose) has been definitely determined (22, 23).

Table 3 shows that the tetrasaccharide IM-4 from experiment 1 consumes two moles of periodate more than the trisaccharide, which intimates that the tetrasaccharide fraction is not homogeneous. The four possible tetrasaccharides (4:1-4:4 in Fig. 1) should consume the following numbers of periodate: 4:1, 16; 4:2, 11; 4:3 12 and 4:4 7 moles. Thus it seems that the tetrasaccharide fraction does not contain large amounts of 4:4 but may possibly contain all three 4:1-4:3.

Experiment 4

In order to obtain more homogenous limit dextrin fractions, a 50 ml unfractionated sample of dextrans (10 g dry weight, average chain length 6.8) was treated with 10 ml saliva for two months. After fermentation and filtering the dextrans (Ib) were fractionated in ordinary way on a carbon-celite column.

Experiment 5

Stability of the various limit dextrin fractions against salivary amylase

To 5% solutions of the fractions were added equal volumes of filtered saliva; the mixtures (pH = 7) were kept under toluene at 30°. Samples were tested by paper chromatography after 1, 2, 4 and 8 days.

Dextrin fractions from Expts. 1, 2 and 4 were used; the following results were obtained.

Trisaccharide fractions. No action of salivary amylase could be noticed.

Tetrasaccharide fractions. Small amounts of glucose could be identified on the paper chromatograms. However, the amounts of tetrasaccharide were not noticeably decreased.

Pentasaccharide fractions. All pentasaccharides disappeared completely. The pentasaccharide fraction from Experiment 1 gave chiefly tetrasaccharides + glucose; small amounts of trisaccharide and maltose were also formed. The pentasaccharide fraction from Experiment 2 gave only tetrasaccharides and glucose, and those from Experiment 4 tetrasaccharides + glucose together with traces of maltose.

Stability of oxidized dextrin fractions against salivary amylase

The tetra- and pentasaccharide fractions from Experiments 2 and 4 were oxidized with potassium hypoiodite in methanol (17). The reducing group of the dextrans is converted to a carboxyl group. The precipitated potassium salts were washed thoroughly with methanol containing traces of thiosulphate to remove all iodine, then with pure methanol. The salts were dissolved in water and the solutions were treated with equal volumes of saliva. In the acids a liberation of glucose can occur only from the non-reducing end-groups of the mother dextrans. The following results were obtained:

Tetrasaccharide fraction. No glucose was formed from the tetrasaccharide fraction from Experiment 2, but small amounts were split off from the tetrasaccharide fraction from Experiment 4.

Pentasaccharide fraction. Glucose was split off from both pentasaccharide fractions investigated, and in addition traces of maltose were formed from the pentasaccharide fraction of Experiment 4.

Discussion and summary

The experiments on the oxidation by periodate and on the action of large amounts of salivary amylase on the various dextrin fractions allow the following conclusions:

The *trisaccharide* fraction is homogeneous and contains only panose (trisaccharide 3:1 in Fig. 1). Since this trisaccharide is probably formed from one or more tetrasaccharides with the liberation of glucose, or possibly from pentasaccharides with the liberation of maltose, it is concluded that the *tetrasaccharide* fraction of the limit dextrans may contain the dextrans 4:1, 4:2 and/or 4:3 but not 4:4. This is in accord with the periodate consumption of the tetrasaccharide fractions. The tetrasaccharide fraction from Experiment 2 probably contains 4:1 alone, since the corresponding carboxylic acid does not yield glucose on treatment with salivary amylase. The tetrasaccharide fractions from Experiments 1 and 4 contain the same oligosaccharide 4:1 but mixed with 4:2 and/or 4:3. The *pentasaccharide* fractions may contain all four isomers shown in Fig. 1.

Large amounts of saliva cause the pentasaccharide fractions to disappear completely, and the main product formed is a tetrasaccharide fraction. Provided that saliva does not contain "limit dextrinases" which convert higher dextrans into tetrasaccharides, it seems that tetrasaccharides may be regarded as the true limit dextrin.

A trisaccharide, panose, is formed, though in comparatively small amounts. Since the tetrasaccharides tested appear to be practically stable against the enzyme used, it may be that the trisaccharide is produced from certain pentasaccharides with the liberation of maltose.

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Studies on yeast invertase

V. Inhibition by Ag^+ and the complex formation between Ag^+ and degradation products of nucleic acids

By KARL MYRBÄCK and EBBA WILLSTAEDT

With 2 figures in the text

The strong inhibition of yeast invertase by silver (and certain other heavy metal) ions is probably due to the presence of sulphhydryl groups in the enzyme protein (1). However, the dependence of the silver inhibition on pH shows that in the equilibrium enzyme- Ag also groups other than sulphhydryl groups must be involved, possibly by influencing the "availability" of the sulphhydryl groups to Ag^+ . An explanation of the pH-dependence of the invertase inhibition by Ag^+ would be the following: At the pH optimum, i.e. in the "isoelectric" enzyme (according to Michaelis), the HS-groups are "masked" and not reacting with the silver ions. In less acid (neutral, alkaline) solution we have an equilibrium between the active form and an inactive (the anion according to Michaelis), the equilibrium being governed by the loss of a proton from a group of the enzyme protein having a pK value of about 6.7. This loss of the proton brings about a structural change in the protein by which the sulphhydryl groups are rendered available to Ag^+ .

Several reasons, e.g. the magnitude of the pK value, seem to suggest that the group(s) responsible for the alkaline branch of the activity-pH-curve are imidazole groups. It is noteworthy that, apart from the sulphur-containing amino acids, histidine appears to be the only one of the common amino acids which has a marked affinity for Ag^+ . It is true that the affinity of histidine for Ag^+ is considerably lower than that of the enzyme protein; however, the equilibrium histidine- Ag shows the same dependence on pH as that of the invertase inhibition by Ag^+ (2). Even if the assumption appears inevitable that the binding of Ag^+ by yeast invertase is mediated principally by sulphhydryl groups, the imidazole groups must be involved in one way or other. The behaviour of imidazole-containing substances with respect to binding of Ag^+ and certain other metal ions is therefore of general interest for the question of the metal ion inhibition of the enzyme.

It has been shown in a preceding paper (3) that among silver-binding impurities in invertase solutions nucleic acids probably are most important. It appears probable that the strong binding of Ag^+ by nucleic acids is mediated by groups of the nitrogenous bases of the nucleotides, possibly the imidazole groups of the purines. It could be shown, however, that the silver-binding capacity of yeast nucleic acid is almost abolished by acid or alkaline or enzymatic hydrolysis of the polynucleotide. This would mean that the presence of a certain base in a

nucleotide unit of a polynucleotide is not enough to explain the high silver-binding capacity of the latter; a favourable position of Ag^+ -binding groups in adjacent base moieties is probably necessary. It should be remembered in this connexion that the histidine-silver complex contains 2 histidine/Ag, and that free imidazole (4) and histamine (1) bind silver much more weakly than histidine.

The conclusion that mononucleotides and other degradation products of nucleic acids have a low silver-binding capacity compared to that of yeast nucleic acid, was drawn on the basis of hydrolysis experiments only, and it appeared necessary to determine the silver-binding of some well-defined components of yeast nucleic acid before entering upon the question of the binding of Ag^+ by the polynucleotide.

Experimental

Materials

Invertase. The invertase solution A6 used in the experiments has been described in a preceding publication (3).

Buffer, sodium acetate - acetic acid; concentration of sodium acetate in the assay mixture 0.01 *N*. The complexing of Ag^+ by the acetate ion is negligible at this concentration.

Adenine (Th. Schuchart, München); *Adenosine*, *Guanosine* and *Uridine* (Hofmann-La Roche, Basel); *Adenosine-3'-phosphate* and *Adenosine triphosphate* (Sigma Chem. Co.); *Adenosine-5'-phosphate* (Schwarz laboratories); *Guanosine-5'-phosphate* (Fluka A.G.); *Cytosine* (Dougherty Chemicals). *Uracil* was prepared synthetically from urea and malic acid in fuming sulphuric acid.

Method

Assay mixture: (a) 4.75 g saccharose, buffer, silver nitrate solution and water to 70 ml. Temperature equilibration at 30°. (b) The substance to be tested was dissolved in 29 ml water and brought to 30°. Solutions (a) and (b) were mixed; 1 min later the reaction was started by the addition of 1 ml invertase solution. The determination of reaction rate has been described earlier (4, 5). In some experiments the protecting substance was incubated with the substrate-buffer-silver solution for a longer time than 1 min. In most cases the same relative activity (RA) was found, irrespective of the incubation time. In a few cases, e.g. with adenine (4), a small increase of RA with incubation time was observed, showing that, in these cases, the binding of Ag^+ , though very rapid, is not quite instantaneous.

All experiments were carried out at pH = 5.3. If necessary, the solutions of the protecting substances were brought to this pH before addition to the substrate-buffer-silver solution.

Results

The relative activity of the enzyme at various Ag^+ concentrations was determined (Fig. 1). An experiment at $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6}$ *N* was carried out in the presence of the protecting substance to be tested, e.g. at an adenine concentration of 2.2×10^{-4} *M*. The relative invertase activity was found to be $\text{RA} = 0.22$. It is seen from Fig. 1 that this RA value corresponds to $[\text{Ag}_{\text{tot}}] = 2.4 \times 10^{-6}$. This means that 2.6×10^{-7} equiv. Ag have been bound by 2.2×10^{-5} moles adenine.

Experiments of this kind were performed with the addition of various protecting substances (Table 1). The amount of silver ion bound was calculated (Fig. 2). It appears that the ability to bind Ag^+ at the concentrations in question varies

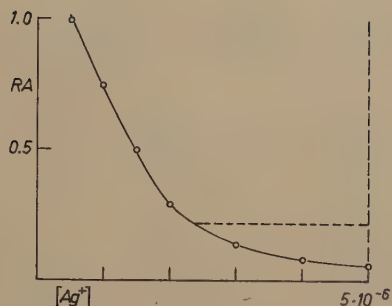


Fig. 1.

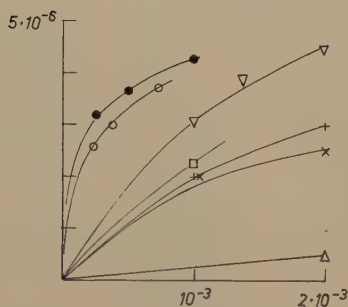


Fig. 2.

Fig. 1. Inhibition curve of invertase A6. The dashed line illustrates calculation of silver bound by added protecting substances, e.g. adenine.

Fig. 2. Binding of Ag^+ by some degradation products of yeast nucleic acid: *Abcissa*, concentration of protecting substance, mole/liter; *ordinate*, equiv. Ag^+ bound per liter solution, $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6} \text{ N}$. ●, Guanosine-5'-phosphate; ○, adenine; ▽, cytosine; □, adenosine-3'-phosphate; +, adenosine-5'-phosphate; ×, guanosine; △, adenosine.

Table 1. Binding of Ag^+ at pH = 5.3 by certain degradation products of yeast nucleic acid.

Protecting substance		Equiv. Ag bound $\times 10^6$	
Name	Mole/liter	RA	per liter
Adenine	2.2×10^{-4}	0.22	2.6
	3.7×10^{-4}	0.30	3.0
	7.4×10^{-4}	0.62	3.75
	3.7×10^{-3}	0.90	4.3
Adenosine	5×10^{-5}	0.06	0
	5×10^{-4}	0.065	0
	10^{-3}	0.06	0
	2×10^{-3}	0.07	< 0.5
Adenosine-3'-phosphate	10^{-3}	0.18	2.3
Adenosine-5'-phosphate	10^{-3}	0.15	2.0
	2×10^{-3}	0.29	3.0
Adenosinetri phosphate	10^{-3}	0.06	0
Guanosine	10^{-3}	0.14	2.0
	2×10^{-3}	0.21	2.5
Guanosine-5'-phosphate	2.5×10^{-4}	0.36	3.2
	5×10^{-4}	0.58	3.7
	10^{-3}	0.86	4.2
Uracil	10^{-3}	0.06	0
	5×10^{-3}	0.06	0
Uridine	2×10^{-3}	0.06	0
Cytosine	10^{-3}	0.31	3.05
	1.37×10^{-3}	0.68	3.9
	2×10^{-3}	1.00	~ 4.5

seemingly irregularly. However, the binding capacity is small compared to that of yeast nucleic acid; the binding of Ag^+ by a commercial preparation of this substance, determined as above, is shown in Table 2, and Fig. 3 shows the very great difference in the binding of Ag^+ by yeast nucleic acid and free adenine.

Table 2. Binding of Ag^+ at pH = 5.3 by yeast nucleic acid.

Concentration is given as the average number of mononucleotide residues per liter.

Yeast nucleic acid mg/l	[Nucleotide residue] $\times 10^5$	RA	Equiv. Ag bound $\times 10^6$ per liter
4.1	1.2	0.15	2.0
7.0	2.1	0.38	3.25
8.2	2.4	0.48	3.5
10.3	3.1	0.70	3.9

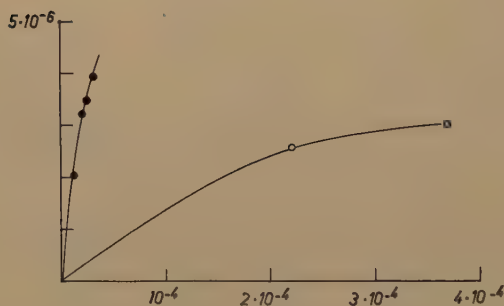


Fig. 3.

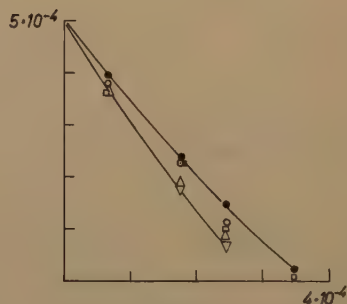


Fig. 4.

Fig. 3. Amount of Ag^+ bound by yeast nucleic acid (●) and by adenine (○), $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-6} N$. *Abscissa*, concentration of protecting substance; *ordinate*, equiv. Ag bound per liter solution.

Fig. 4. Concentration of free Ag^+ ion ($[\text{Ag}_{\text{tot}}] = 5 \times 10^{-4} N$) in the presence of adenine: *Abscissa*, concentration of adenine, mole/l; *ordinate*, concentration of free Ag^+ ion. ●, pH = 3.58; ○, pH = 4.50; □, pH = 5.30; △, pH = 5.95; ▽, pH = 6.80.

It may be mentioned in passing that the ability of the substances tested to bind Ag^+ is not dependent on the presence of the imidazole group: for instance, cytosine binds Ag^+ nearly as strongly as does adenine or guanosine-5'-phosphate (the low solubility of guanine prevented determination of its silver-binding capacity). On the other hand, since uracil does not bind measurable quantities of silver, an amino group on the pyrimidine ring is required for the complexing of Ag^+ .

The binding of Ag^+ by adenine and its dependence on the pH was also determined electrometrically (2). The results (Table 3) show that at the silver ion concentration used, $[\text{Ag}_{\text{tot}}] = 5 \times 10^{-4} N$, adenine binds Ag^+ strongly; the curves of Fig. 4 are not far from straight lines. The figure also shows that the binding of Ag^+ varies comparatively little with pH; however, the silver-binding capacity increases somewhat with increasing pH. The same was found earlier in experiments on the protecting action of adenine on the silver inhibition of invertase (4). The binding of silver by histidine varies much more strongly with the pH (2).

Table 3. Electrometrical determination of free Ag^+ in the presence of adenine at various pH values.

$$[\text{Ag}_{\text{tot}}] = 5 \times 10^{-4} N.$$

pH	Adenine, mole/l	$[\text{Ag}_{\text{free}}^+]$
3.58	—	5×10^{-4}
	7×10^{-5}	3.94×10^{-4}
	1.75×10^{-4}	2.40×10^{-4}
4.50	2.45×10^{-4}	1.48×10^{-4}
	7×10^{-5}	3.78×10^{-4}
	1.75×10^{-4}	2.26×10^{-4}
	2.45×10^{-4}	1.14×10^{-4}
5.30	3.50×10^{-4}	2.30×10^{-5}
	7×10^{-5}	3.62×10^{-4}
	1.75×10^{-4}	2.26×10^{-4}
	2.45×10^{-4}	1.00×10^{-4}
5.95	3.50×10^{-4}	6.67×10^{-6}
	7×10^{-5}	3.70×10^{-4}
	1.75×10^{-4}	1.86×10^{-4}
	2.45×10^{-4}	8.30×10^{-5}
6.80	1.75×10^{-4}	1.86×10^{-4}
	2.45×10^{-4}	7.10×10^{-5}

SUMMARY

The silver-binding capacity of a number of ribonucleic acid degradation products in very diluted solutions has been determined by measuring their protecting action on silver-inhibited yeast invertase. Some of the substances tested do not bind measurable quantities of Ag^+ at the concentrations investigated, but others, purine as well as pyrimidine derivatives, have a distinct silver-binding capacity. However, all substances investigated bind Ag^+ weakly compared to yeast ribonucleic acid (the "concentration" of ribonucleic acid being calculated as the number of mononucleotide units per liter).

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Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

N-Oxyde von Aminosäuren und Purinen

Verhalten von Nukleinsäuren und Nukleoproteiden gegen Hydroperoxyd und gegen Reduktone

Von HANS VON EULER und HANS HASSELQUIST

Zur Kenntnis der schon zu Anfang dieses Jahrhunderts (siehe J. MEISENHEIMER (1)) bekannten Klasse der N-Oxyde haben japanische Forscher, besonders E. OCHIAI u. Mitarb. (2), T. OKAMOTO (J. Pharm. Soc. Japan 71, 297) u. a. wesentlich beigetragen. In letzter Zeit haben die N-Oxyde wieder erhebliches Interesse gewonnen durch die Entdeckungen und Beobachtungen von T. YOSHIDA (3) und ISHIDATE (4) sowie von DRUCKREY (5) über die chemotherapeutische Rolle von N-Oxyd-Lost bei der Krebs-Heilung sowie durch die Arbeiten von N. BROCK (6) und ARNOLD (7) über den N-Lost-Phosphamidester B 518 (Mitomen, Asta), schliesslich durch den Nachweis der cancerogenen Wirkungen von N-Oxyd-Lost durch STEINHOFF und BOK TSCHIN KUK (8).

Die Arbeiten von OCHIAI betrafen zunächst hauptsächlich Chinolin- und Nitrochinolin-Derivate, deren anti-canceröser Effekt von FUKUOKA (9) studiert wurde.

Darstellung neuer N-Oxyde

Die hier mitzuteilenden Versuche hatten nicht das Ziel, weitere N-Oxyde darzustellen, welche wie die N-Oxyd-Loste die Krebsentwicklung beeinflussen. Vielmehr erschien es uns als eine wichtige biochemische Aufgabe, festzustellen, *ob und unter welchen Konzentrationsbedingungen das endogen auftretende Hydroperoxyd die intermediäre Bildung von N-Oxyden und ihre Teilnahme am Stoffwechsel veranlasst.*

In einer vorhergehenden Mitteilung (dieses Arkiv, 12, 48; 1958) wurde das bisher nicht bekannte *Nicotinsäureamid-N-Oxyd* $C_6H_6O_2N_2$, Schmp. 287–288° kurz beschrieben. Es fungiert, wie das Ausgangsmaterial, das Nicotinsäureamid, als wachstumsförderndes Vitamin. Seine Stabilität und den Einfluss einer ev. Blockierung des Nicotinsäureamides durch den N-Oxyd-Sauerstoff im Stoffwechsel haben wir noch nicht untersucht.

Wir haben nun, in Fortsetzung dieser Untersuchung über N-Oxyd-Bildung, die als Aktivator (10) in vielen Hinsichten wichtige Aminosäure *Histidin* und das Purinderivat *Adenin* mit Hydroperoxyd reagieren lassen.

Darstellung des L-Histidin-N-Oxyds

1 g L-Histidin (freie Base) in 20 ml Eisessig gelöst, wurde mit 10 ml 30%iger Hydroperoxyd-Lösung versetzt, und 48 Stunden auf 65° erwärmt. Gelbfärbung trat unter der Reaktion nicht ein. Die ganze Reaktionslösung wurde im Vakuum zur

Trockne verdampft, wodurch eine zum Teil schmierige Kristallmasse erhalten wurde, die in etwas siedendem Methanol gelöst zur Kristallisation gebracht wurde. Dabei bildeten sich farblose, nadelförmige Kristalle, die abfiltriert und mit wenig Methanol gewaschen wurden. Ausbeute 550 mg + weitere 200 mg aus der Mutterlauge.

Durch Umkristallisieren der beiden Fraktionen aus Methanol entstand eine kompakte, schwach gefärbte Kristallmasse, die bei 260° unter starker Zersetzung schmolz.

Zur Untersuchung des Reinheitsgrades des erhaltenen Produktes wurde dasselbe papierchromatographisch untersucht.

Dabei kam zur Verwendung das Papier Munktell OB; die Flüssigkeit 77% n-Butanol, 13% Wasser, 10% Ameisensäure.

Entwicklung durch eine diazotierte Sulfanilamid-Lösung, hierauf Bespritzen mit Natriumcarbonat-Lösung. Hierdurch treten Imidazole mit Rosa-Farbe hervor (D. BOLLING u. R. J. BLOCK, *Feder. Proc.* 8, 185; 1949).

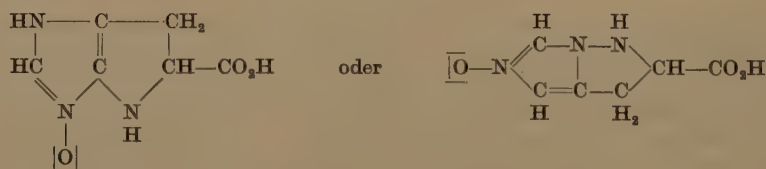
Bei der Untersuchung der Subst. vom Schmp. 260° zeigte sich, dass dieselbe nur Spuren von L-Histidin mit $R_f = 0,01$ enthielt. Ausserdem entstand ein sehr deutlicher rosa Farbflecken mit $R_f = 0,30$.

Die Subst. 281 wurde nun noch zweimal aus Methanol umkristallisiert, und wurde rein erhalten mit dem Schmp. 265° (starke Zersetzung).

Subst. 281. Ber. für $C_8H_7O_3N_3$	C 42,60 %	H 4,17 %	N 24,81 %
Gef.	42,39	3,88	24,81

Die Subst. ist äusserst leicht löslich in Wasser und in Eisessig, schwer löslich in Aceton, Chloroform, Äther und Benzol, ziemlich löslich in warmem Methanol und Äthanol.

Die Subst. 281 ist also, wie zu erwarten war, kein Mono-N-Oxyd des L-Histidins, vielmehr deuten die Analysen-Resultate darauf hin, dass unter Wasser-Abspaltung eine Ringbildung eintritt, etwa nach dem Schema



Durch die Histidase der Leber wird Histidin bis zur Glutaminsäure abgebaut. Der grösste Teil des Histidins ist in Peptidketten eingebaut, u. zw. nach A. KOSSEL in der Weise, dass der Guanidinrest frei bleibt. Serum-Albumin enthält 4% Histidin.

Mit Zn und Co bildet Histidin Chelate. Die Imidazolgruppe des Histidins gibt Anlass zur Bildung eines Ag-Salzes. Histidin konnte als endständige Aminosäure in Proteinen noch nicht nachgewiesen werden.

Im Anschluss an die Darstellung von L-Histidin-N-Oxyd wurde auch die Einwirkung von Hydroperoxyd auf *Histamin* und auf *Tryptophan* untersucht.

Nach 24stündiger Einwirkung von 3%iger Hydroperoxyd-Lösung auf Histamin bzw. Tryptophan wurden bei der Papierchromatographie (Entwicklung durch Ninhydrin) deutliche Effekte gefunden. Das N-Oxyd des Histamins wanderte schneller als Histamin, das N-Oxyd des Tryptophans wanderte dagegen wesentlich langsamer als das Tryptophan.

Auf N-O-Derivate anderer heterocyclischer Ringsysteme (Derivate von Sulfa-pyridin und Sulfathiazol und vermutlich analoge Derivate von Methämoglobin) kommen wir an anderer Stelle zurück.

Über die Reaktion von *Nucleinsäuren* mit Hydroperoxyd ist folgender Versuch mitzuteilen:

0,5 g Hefenucleinsäure wurde mit 10 ml Eisessig und 5 ml einer 30%igen H_2O_2 -Lösung 48 Stunden auf 60° erwärmt. Durch Eindunsten im Vakuum zur Trockne wurde eine farblose schmierige Masse erhalten, die sich — im Gegensatz zum Ausgangsmaterial — in Wasser ziemlich leicht löste.

Ein kleiner Teil des Produktes wurde papierchromatographisch untersucht. Dabei konnten keine niedrig molekularen Bruchstücke nachgewiesen werden, auch zeigte sich kein Unterschied in der Wanderungsgeschwindigkeit zwischen der behandelten und der unbehandelten Nucleinsäure. Die behandelte Nucleinsäure konnte aus einer ziemlich konzentrierten wässrigen Lösung durch Zusatz von Methanol oder Aceton ausgefällt werden und wurde als farbloses, ziemlich festes Pulver erhalten.

Darstellung des Adenin-N-Oxyds

1 g Adenin in 20 ml Eisessig wurde mit 10 ml einer 30%igen Hydroperoxyd-Lösung 48 Stunden auf 65° erwärmt, wobei die anfangs farblose Lösung sich citronengelb färbte. Die Reaktionsmischung wurde sodann im Vakuum bis auf ein kleines Volumen eingengt, wobei ein Produkt in Form kleiner Blättchen auskristallisierte. Nach Zusatz von etwas Eisessig wurden die Kristalle abfiltriert und gewaschen. Ausbeute 0,8. Nach zweimaligem Umkristallisieren aus wenig Eisessig erhielten wir farblose Blättchen vom Schmp. 300° . Dieselben wurden papierchromatographisch untersucht.

Papier Munktel OB. Lösung: 77% n-Butanol, 13% Wasser, 10%.

Entwicklung durch Bespritzen mit einer $Hg(NO_3)_2$ -Ameisensäure-Lösung, hierauf Waschen mit verd. Salpetersäure, Wasser und zuletzt mit Ammoniumsulfid-Lösung. Graue Flecken zeigen Adenin oder Adenin-Derivate an. (E. FISCHER und E. CHARGAFF (11)).

Das Adenin selbst (Ausgangs-Subst.) ergab $R_f = 0,17$. Sbst. 282 ergab $R_f = 0,27$.

Analyse.

Sbst. 282.	Ber. für $C_6H_5N_5O$ Adenin-mono-N-Oxyd.	C 39,73 %	H 3,34 %	N 46,34
Gef.	—	40,32	3,95	45,32

Die Sbst. 282 erwies sich als Adenin-mono-N-oxyd. Sie ist ziemlich leicht löslich in Wasser zum Unterschied von Adenin; ziemlich leicht löslich in Eisessig, schwerlöslich in übrigen organischen Lösungsmitteln. Siehe auch: M. A. STEVENS *et al.*, J. Am. Chem. Soc. 80, 2755 (1958).

Ein in der Fig. 1 dargestellter Versuch wurde ausgeführt um festzustellen, ob die Bildung von N-Oxyden auch unter physiologischen Bedingungen eintritt. Dies ist jedenfalls bei gewissen Substanzen der Fall. Der in Fig. 1 mitgeteilte Versuch wurde mit 0,002%iger Hydroperoxydlösung ausgeführt, was durchaus in vivo vorkommenden Konzentrationen entspricht. Wir sind daher damit beschäftigt, N-Oxyde aus frischen Organen zu isolieren. Über N-Oxyde von Adenosin und ATP berichten wir an anderer Stelle.

Unter den Aminosäuren bilden nur die wenigen ein N-Oxyd, welche — wie Histidin und Tryptophan — einen Imidazol-, Indol- oder anderen heterocyclischen Ring ent-

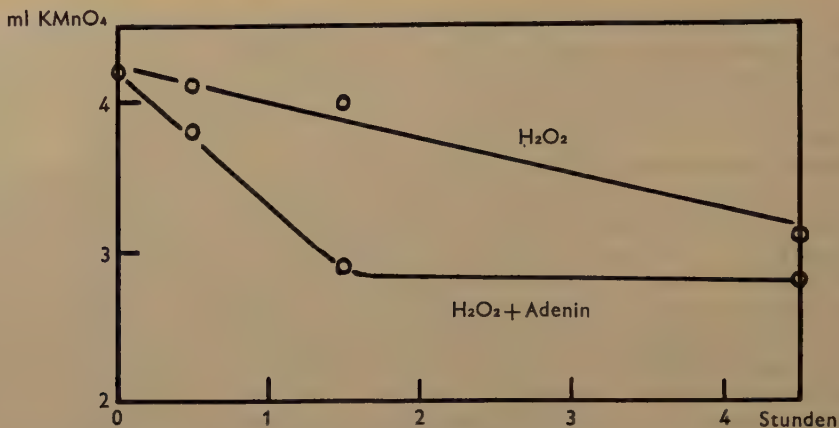


Fig. 1. 45 mol 0,002%iges H₂O₂ + 5 ml H₂O. 45 ml 0,002%iges H₂O₂ + 5 ml H₂O + 10 mg Adenin. Temp. 37°.

halten. Es wurde geprüft, ob Proteine und Proteide, welche diese Aminosäuren als endständige Glieder enthalten, ebenfalls zu N-Oxyd-Bildung befähigt sind.

Im Verlauf dieser Arbeiten wurden auch *Kollagen*¹ sowie die Rohmaterialien *Seide*² und Wolle in den Kreis der Versuche gezogen, Kollagen wegen seines Histidin-Gehaltes, Seide bzw. Seidenfibroin wegen des Tryptophan-Gehaltes.

Wolle ist von J. B. SPEAKMAN, Universität Leeds, in ausgedehnten Untersuchungen bearbeitet worden (12), das Verhalten der Wolle gegen Reduktionsmittel, besonders gegen Trios-Redukton von J. R. HOLKER (13) in seiner Dissertations-Schrift aus dem SPEAKMANSchen Institut.

Ohne in das Gebiet des SPEAKMANSchen Arbeitskreises eingreifen zu wollen, gehen wir nur kurz auf zwei Fragen ein, die mit unseren früheren Erfahrungen in Zusammenhang stehen, nämlich:

- 1) ob die Seide bzw. das Seidenfibroin in ihrem Verhalten zu Reduktionsmitteln Analogien mit der Wolle zeigen,
- 2) Ob die Reaktion von Wolle (vgl. HOLKER) und Seide gegen Triose-Redukton für dieses spezifisch ist oder ob ein solches Verhalten allen Reduktonen oder wenigstens allen Aci-Reduktonen zukommt.

Was die erste Frage betrifft, so zeigt die Figur 2, die sich auf Triose-Redukton bezieht, dass in der Redukton-Lösung das Redukton in Gegenwart von Wolle schneller verschwindet als in der Gegenwart der gleichen Menge Seide.

Über das Verhalten der Wolle in Redukton-Lösung gibt der folgende Versuch (Tabelle 1) Auskunft, bei dem die Reduktonlösung — es wurden von Zeit zu Zeit je 0,2 ml entnommen — mit einer Lösung von Tillmans Reagens (TR), Dichlorphenol-Indophenol) titriert wurde.

¹ Für Überlassung von Material und für wertvolle Informationen und Ratschläge sind wir Herrn Prof. K. H. Gustavson, Forschungs-Institut der schwed. Gerberei-Industrie, sehr zu Dank verpflichtet. Siehe K. H. GUSTAVSON in: *Connective Tissue*, Scientific Publications, Oxford, 1957, 185. — Ebenda: I. BANGA u. J. BALÓ, p. 254.

² Wir verdanken Herrn Professor K. YAMAFUJI, Agricultur-chem. Institut der Universität Fukuoka, Proben reiner japanischer Seide aus Bombyx.

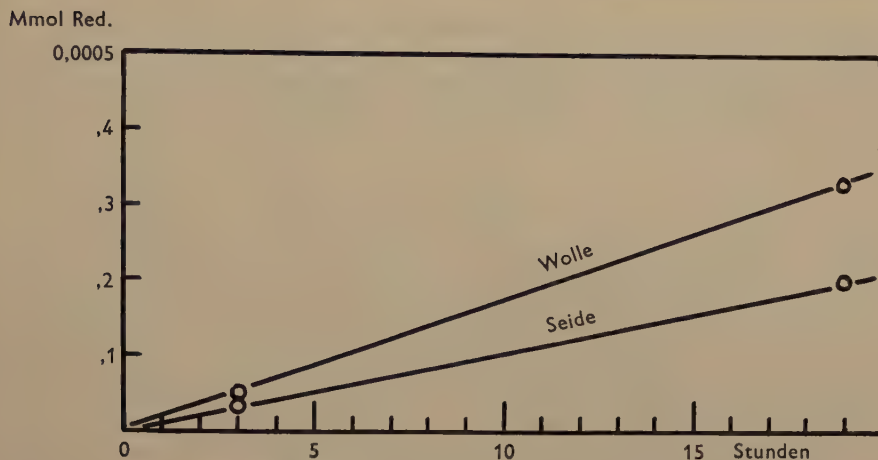


Fig. 2. Reduktion-Verbrauch durch 1 mg Fiber.

Tabelle 1. Versuch.

Grundlösung: 100 mg Redukton in 12,5 ml Wasser. 49 mg Wolle in 5 ml Reduktonlösung, enthaltend 40 mg Redukton. Reaktionszeit 20°.

	0 Stunden	3 Stunden	19 Stunden
TR Verbrauch, Wasser	4,7 ml	4,5 ml	3,4 ml
TR-Verbrauch in Gegenwart von Wolle	0 ml	0,2 ml	1,3 ml
Verbrauch in Gegenwart v. Wolle			11,2
Gewicht der Wolle	49 mg		59,9 mg
Gewichtszunahme der Wolle			10,9 mg
Substanz-Menge in der Lösung	40,0 mg		18,0 mg
Reduktonmenge in der Lösung	40,0		18,0 mg

Der Verbrauch von Redukton durch Seide geht aus folgenden Zahlen hervor:

Ursprüngliche Reduktonmenge	40,0 mg
Redukton-Verbrauch durch die Seide	6,9 mg
Nach der Reaktion in der Lösung gefundene Substanzmenge	20,8 mg
Differenz durch Trocknung	9,3 mg
Summe	37,0 mg
Nicht identifizierte Differenz.	3,0 mg

Das Aci-Redukton (14) Ascorbinsäure (AS) verhält sich sowohl gegen Seide wie gegen Wolle inaktiv wie die Tabelle 2 zeigt. Siehe auch Tabelle 3.

Wägung des Versuchs-Rückstandes der eingedunsteten Lösung konnte im Dioxyfumarsäure, DFS-Versuch nicht ausgeführt werden, da ein starker Zerfall der DFS in CO₂ und Glykolaldehyd eintrat.

Die obige zweite Frage (S. 188) ist also dahin zu beantworten, dass auch andere Reduktone als Triose-Redukton eine gewisse Einwirkung auf Seide und Wolle besitzen, dass aber auch so ausgesprochene Aci-Reduktone wie Ascorbinsäure sich als inaktiv erweisen. In dieser Hinsicht muss das Versuchsmaterial noch erweitert

Tabelle 2.

Reaktions- zeit	Verbraucht ml TR-Lösung (20°)		
	Wolle + AS	Seide + AS	AS
0 Stunden	16,3	16,5	16,4
19 „	14,5	14,9	14,8
45 „	13,5	13,9	13,9

	50 mg Fiber. Gewichts- erhöhung	50 mg Fiber. Von Fibern auf- genommenes Gew. vom reinen Redukton	Von der Lös. zurückge- wonnene Substanz	Summe
Wolle	10,9	11,2	27,3	38,5
Seide	8,2	6,9	30,1	37,0

Tabelle 3. Einwirkung von Dioxyfumarsäure, DFS, auf Seide bzw. Wolle.

Reaktionslös.: 5 ml Dioxyfumarsäurelös., 37 mg/5 ml. 51,2 mg Seide bzw. 50,4 mg Wolle. Zur TR-Titration je 0,2 ml entnommen. Reaktionszeit 20°.

	0 Stunden	5,5 Stunden	24 Stunden
Korr. Verbrauch von TR	3,7 ml	2,5 ml	2,8 ml
Verbrauch von DFS in Gegenwart von Seide	0	1,2 ml	0,9 ml
Verbrauch von DFS in Gegenwart von Seide			9,0 mg total
Gewicht der Seide	51,2 mg		53,4 mg
Gewichtszunahme der Seide			2,2 mg
Korr. Verbrauch von TR	3,7 ml	2,6 ml	2,9 ml
Verbrauch von DFS in Gegenwart von Wolle	0	1,1 ml	0,8 ml
Verbrauch von DFS in Gegenwart von Wolle			8,0 mg total
Gewicht der Wolle	50,4 mg		54,1 mg
Gewichtszunahme der Wolle			3,7 mg

werden, was im Einverständnis mit Prof. J. B. SPEAKMAN und Prof. K. H. GUSTAVSON geschehen soll. Kollagen interessiert uns auch wegen seiner Beziehungen zu Mucinen, die durch Reduktone gelöst werden (Arkiv 8, nr 37 und 9, nr 12).

Eine Bildung von *N-Oxyden aus Nucleinsäuren* durch Einwirkung von Stickstofflost-N-Oxyd bzw. Mitomen (B 518), Asta) haben wir nicht beobachten können. Auch auf die Kondensation von Seide und Wolle hat Mitomen keinen Einfluss.

Nach CHR. SCHOLTISSE (15), der im Institut von H. LETTRÉ früher die Einwirkung von Stickstofflost auf Serumeiweiss studiert hat, wird die Wanderung von Nucleinsäuren bei der Papier-Elektrophorese gegen den positiven Pol durch Stickstofflost und durch Stickstofflost-N-Oxyd (in Gegenwart von Colchicin) *vermindert*. Die Fähigkeit von Nucleinsäuren mit Methylgrün-Pyronin zu färben, verschwindet nach der Reaktion mit Stickstofflost bzw. Stickstofflost-N-Oxyd. Die Reaktionen von

N-Lost und N-Lost-N-Oxyd gegen Modells substanzen, wie z. B. Adenin und Adenosin, erzeugten zahlreiche Produkte (vermutlich durch Substitution im Purinkern).

Nach J. A. V. BUTLER *et al.* (16) wird Desoxyribonucleinsäure durch Behandlung mit Stoffen vom Stickstofflost-Typus abgebaut; das Molekulargewicht fällt von ca. 10^6 bis auf Werte zwischen 10^4 und 10^5 .

Darstellung eines Bis-N-Oxydes

Bis-N-Oxyde waren bis jetzt nicht bekannt. Als Ausgangsmaterial für ein Bis-N-Oxyd haben wir *p*-Phenanthrolin verwendet, das in Annäherung an das Verfahren von A. KAUFMANN u. R. RADOSEVIČ (Ber. 42, 2613; 1909) bereitet wurde.

10 g 6-Amino-chinolin, 20 g Glycerin, 7,75 g Diarsenik-pentoxyd und 24 g konz. Schwefelsäure wurden auf 150° erwärmt, bis die Blasenbildung nach 3 Stunden aufgehört hatte. Die nun homogene Lösung wurde mit wenig Wasser versetzt und mit konz. Kalilauge alkalisiert. Die Reaktionslösung wurde in einen Scheidetrichter übergeführt und mit Chloroform zweimal extrahiert. Die vereinten Chloroform-extrakte wurden mit wasserfreiem Natriumsulfat getrocknet, filtriert und im Vakuum zur Trockne verdampft. Die zurückbleibende Kristallmasse wurde aus Ligroin umkristallisiert, wodurch farblose Nadeln erhalten wurden, die aus Benzol umkristallisiert, entsprechend der Angabe von KAUFMANN u. Mitarb., bei 177° schmolzen.

Umsatz von *p*-Phenanthrolin mit Benzoeperensäure

0,9 g Phenanthrolin wurden mit Benzoeperensäurelösung (Chloroform) in grossem Überschuss — etwa dem 10fachen der für die Bildung eines Bis-N-Oxydes berechneten Menge — versetzt. Dabei löste sich das Phenanthrolin mit schwach gelber Farbe, die allmählich zunahm. Die ganze Reaktionsmischung stand bei Zimmertemperatur einen Tag lang, wobei das Reaktionsprodukt in Form von gelbroten kompakten Kristallen vom Schmp. 279° ausgeschieden wurde.

Die Substanz 280 wurde aus Methanol umkristallisiert und bildete kleine, schwach graugelb gefärbte Nadeln vom unveränderten Schmp. 279° . Ausbeute an reiner Subst. 0,1 g.

Analyse.	Ber. für $C_{12}H_8N_2O_2$	C 67,32 %	H 3,80 %	N 13,20 %
Gef.		67,50	4,06	13,26

Die Substanz 280 erwies sich also als Bis-N-Oxyd. Sie ist in allen üblichen organischen Lösungsmitteln sowie in Wasser ziemlich schwer löslich.

Herrn Ingeniör OSKAR HEIDENBERGER danken wir für wertvolle Hilfe.

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Osmotic measurements on cellulose in diluted cupriethylenediamine solutions

BY HANS VINK

With 4 figures in the text

In a previous work (1) it was shown that CED solutions of cellulose could be extensively diluted with water without the cellulose being precipitated, and that the resulting solutions, although metastable, could be used for physico-chemical measurements. The solvent in these solutions no longer dissolves cellulose and it was reported that they could be dialysed through viscose membranes. This immediately suggests the use of such membranes for osmotic measurements with these solutions. Thus it would be possible to determine the number-average molecular weight of cellulose directly, without first converting it into a derivative.

However, due to the metastable nature of the solutions and to complex formation between cellulose and the solvent and, also, due to the possibility that some of the cellulose of the membrane might dissolve in the CED solution, the situation is rather complicated and some unpredicted effects might influence the results. It was therefore considered necessary to have an empirical check on the results obtained. For this reason measurements were made with cellulose fractions obtained from cellulose acetate fractions through deacetylation, precluding degradation. The DP-values of the cellulose acetate fractions were determined osmotically in acetone. Thus a check of the obtained molecular weights was possible.

In this connection it should be mentioned that osmotic measurements in undiluted CED solutions, using membranes resistant to the solvent, have previously been reported (2). However, it is difficult to prepare suitable membranes and this, together with the fact that concentrated CED solutions are somewhat unstable, makes this approach quite difficult.

The preliminary experiments were performed with ordinary capillary osmometers, but did not give reproducible results. This seemed to be due to variations in the capillary forces and the reason for this was probably precipitation of cellulose at the solution-air interface in the capillary.

It was therefore decided to use an osmometer with an organic liquid manometer. The character of the solvent (strongly alkaline) ruled out all constructions that made use of greased stopcocks. A further requirement was that it should be possible to exclude oxygen from the osmometer during filling and measurement, and of course, it should be easy to handle. As no osmometer described in the literature seemed to fulfil these conditions, a new osmometer was constructed.



Fig. 1

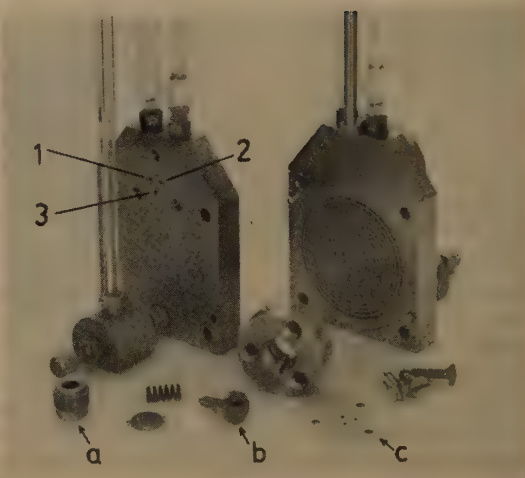


Fig. 2

Fig. 1. Photograph of the osmometer.

Fig. 2. Detailed picture of the osmometer. (a) the tightening screw; (b) the moving part of the valve with the groove visible; (c) the teflon packing. 1, 2 and 3 designate channels to the filling tube, capillary and osmotic cell, respectively. The osmometer was built of stainless steel.

Construction of the osmometer

The osmometer is essentially a Fuoss-Mead block-osmometer which is provided with an organic liquid manometer. The latter is so constructed that the solvent, solution-organic liquid boundaries are located in wide tubes while the air-organic liquid interfaces are situated in the capillaries. Errors due to capillary forces are thus minimized. The manometer is connected to the osmometer through 3-way valves that are set in different positions when filling the osmometer and when making measurements with it. The 3-way valve is provided with a screw that makes it possible to apply pressure on a packing in the valve, thus making leakage impossible. The osmometer is filled through side tubes that are provided with containers for solution and solvent, respectively. In the lower parts of the filling tubes there are needle valves. They are provided with rubber o-ring packings to prevent leakage. These valves are also useful in adjusting the liquid levels in the manometer capillaries. An overall view of the osmometer is given in Fig. 1. Details are given in Fig. 2.

The osmometer is filled in the following way. Solution and solvent are poured into the respective containers and the oxygen removed by evacuation. With the

3-way valves connecting channels 1 and 3 (see Fig. 2) the needle valves are opened and the osmometer is flushed through with fairly large volumes of liquid. Then the 3-way valves are set to connect channels 1 and 2, the solvent and the solution are removed from their respective channels with a syringe and filled with the organic liquid used in the manometer. The valves are then switched to connect channels 2 and 3 and tightened by turning the screws and then the solvent respectively solution-organic liquid boundaries are adjusted to the middle of the wide tubes beneath the capillaries. Final adjustments of the liquid levels in the capillaries are made by means of the needle valves.

Experimental

The cellulose acetate used was a secondary cellulose acetate sample from the British Celanese Company. This was fractionated in acetone using ethyl alcohol as the precipitant (3). During the first fractionation seven fractions were obtained. Of these the second, fourth and sixth were refractionated into three approximately equal fractions of which only the middle fractions were used in further work. Here they will be designated by 1, 2 and 3, No. 1 having the highest DP.

A part of each fraction was deacetylated using sodium-containing methanol in an atmosphere of nitrogen (4-6). For a sample of about 10 g of cellulose acetate 500 ml of methanol with about 2 g of sodium added was used. With continuous stirring the reaction was allowed to proceed at room temperature for 5 hours. The cellulose was then washed with water acidified with acetic acid, then with pure water and finally with acetone. The samples were dried in air at 60°C. Under these conditions complete deacetylation with practically no degradation is effected. Thus cellulose fractions were obtained that had practically the same DP as the corresponding cellulose acetate fractions. The molecular weights of the cellulose acetate fractions were determined in acetone with a Zimm-Meyerson osmometer. The membranes used were Ultracellafilter "allerfeinst".

For osmotic measurements in CED solutions commercial viscose membranes ("sausage-casing" from The Visking Corp., Chicago) were used. The membranes were first soaked in water for several days before mounting in the osmometer. Conditioning of the membrane with CED was performed in the osmometer. Most of the measurements were carried out with solutions having a CED concentration of 0.5/12 *M* (1). Measurements were also made with more concentrated solutions. Solutions with 0.5/7 *M* CED could still be used, but the mechanical strength of the membranes had diminished at this concentration and therefore lower concentrations were preferred.

As the manometer liquid at first 2,2,4-trimethyl-pentane was used. This was easily obtained but turned out to be somewhat too volatile (data: B.p. 99°C, $\rho_{20} = 0.6818$ g/cm³, and $\gamma_{20} = 18.0$ dyn/cm). Later *n*-dodecane was used (data: B.p. 214.5°C, $\rho_{20} = 0.7488$, and $\gamma_{20} = 25.4$ dyn/cm).

During measurements the osmometer was placed in a thermostat and readings were made with a cathetometer. Readings had to be made for four menisci: the two air-organic liquid interfaces in the capillaries and the two solvent, solution-organic liquid interfaces in the wide tubes. Assuming that the cathetometer readings for the two osmometer sides are a_1 , a_2 and b_1 , b_2 , respectively (index 1 refers to the menisci in the capillaries) the excess pressure on the "a-side" expressed as the height of an equivalent manometer-liquid column is given by the following formula:

$$\pi = (a_1 - b_1) + \frac{\rho - \rho_0}{\rho_0} (a_2 - b_2),$$

where ρ is the density of the solution, and ρ_0 the density of the manometer liquid.

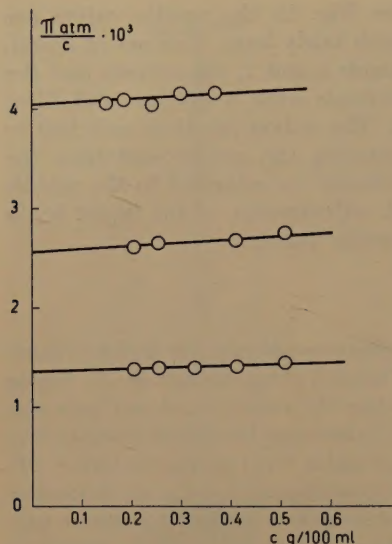


Fig. 3

Fig. 3. Reduced osmotic pressure as a function of concentration for the cellulose acetate fractions in acetone at 20°C.

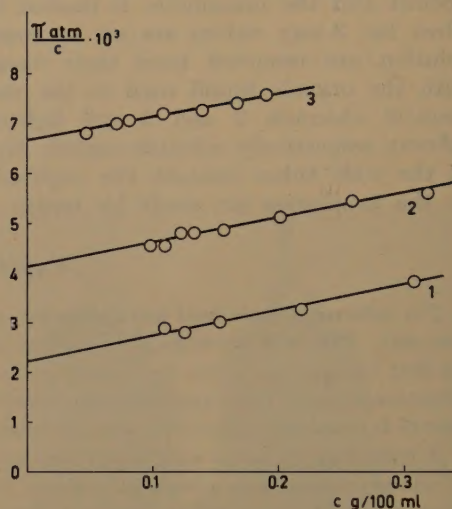


Fig. 4

Fig. 4. Reduced osmotic pressure as a function of concentration for the cellulose fractions at 20°C. Fractions 3 and 2 were measured in 0.5/12 *M* CED, fraction 1 in 0.5/7 *M* CED.

The osmotic pressure was determined by the equilibrium method. With the membranes and capillaries ($d=0.61 \text{ mm}$) used the half-time for approach to equilibrium was of the order of 5 hours and, practically, equilibrium was reached in 1 or 2 days, depending on how far from the equilibrium position the menisci were at the beginning of the experiment. The equilibrium position was quite stable and usually remained within a few per cent of the original position for a period of over a week.

Results

In Table 1 the results of the measurements on the cellulose acetate and the corresponding cellulose fractions are given. In Figs. 3 and 4 the plots of the reduced osmotic pressure against concentration are shown.

The agreement between the DP-values for the cellulose acetate and the corresponding cellulose fractions in Table 1 is seen to be good. It can therefore be concluded that the osmotic measurements on diluted cellulose-CED solutions give correct values for the DP of the dissolved cellulose.

The slopes of the reduced osmotic pressure-concentration lines in Fig. 4 are rather high, thus making an accurate extrapolation to zero concentration difficult. Partly because of this fact the overall accuracy of the measurements in CED is somewhat less than of the corresponding measurements in acetone. Nevertheless this gives a rapid and direct method for determination of the number-average degrees of polymerisation of cellulose samples up to a DP of about 1000.

Table 1. Results of measurements on cellulose acetate and cellulose fractions.

	Fraction No.		
	1	2	3
<i>Cellulose acetate</i>			
Acetyl value ⁽¹⁾	53.4 %	52.7 %	53.2 %
$(\pi/c)_0$ atm.dl.g ⁻¹ (20°C)	1.35×10^{-3}	2.54×10^{-3}	4.06×10^{-3}
Molecular weight	178,000	94,600	59,200
DP	690	367	230
<i>Cellulose</i>			
$(\pi/c)_0$ atm.dl.g ⁻¹ (20°C)	2.20×10^{-3}	4.10×10^{-3}	6.65×10^{-3}
Molecular weight	109,000	58,600	36,200
DP	675	362	223
$[\eta]$ in CED (25°C)	3.47	2.10	1.46
DP _v ⁽²⁾	680	390	257

¹ The acetyl values are given as per cent acetic acid of the cellulose acetate and they were determined according to an alcoholic alkali method (7).

² Calculated according to the equation $[\eta] = 0.0098 \cdot \text{DP}^{0.9}$ (1).

SUMMARY

This investigation deals with osmotic measurements on cellulose in diluted cupriethylenediamine solutions. For this purpose an osmometer with an organic liquid manometer was constructed and measurements were made with cellulose fractions regenerated from cellulose acetate fractions. The DP's of the cellulose acetate fractions were determined osmotically in acetone and were found to be in good agreement with the DP values obtained for the corresponding cellulose fractions in CED.

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